PRODUCTION OF HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS IN RABBITS BY FEEDING DIFFERENT FATS WITHOUT SUPPLEMENTARY CHOLESTEROL

by

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Chapter I

INTRODUCTION

In recent years clinical investigations have produced evidence that the composition and nature of dietary fats have a profound influence on the concentration of the serum lipids. This has introduced new aspects in the discussion of the rôle played by the diet in the development of human arteriosclerosis. Clinical trials in various countries have shown that vegetable and marine oils rich in polyunsaturated fatty acids depress the serum cholesterol level in normals and in patients with hypercholesterolemia, while fats poor in these acids, such as animal fats and hydrogenated vegetable oils, enhance the cholesterol level in the blood. The total amount of fat ingested as well as the cholesterol content of the diet appears to be of subordinate importance in this respect.

These investigations have usually been concerned only with the serum cholesterol level and have not proved whether the enhancement of the serum cholesterol is followed by arteriosclerotic changes. No clinical method is available to yield direct evidence of such changes. Such investigations would require many years observation under rigidly standardized conditions and large groups in which the vessels could be studied post mortem. It would also be difficult to secure an acceptable control group, since most people in this country dying in middle age and later most probably have atheromatous changes in the aorta.

Therefore, in the investigation of dietary fats for any atherogenic properties, we must resort to animal experiments in which the conditions may be controlled for a relatively long time and which permit the study of one variable at a time. In the investigations that have been carried out at the Department of Medicine, University of Lund, on the effect of different fats, rabbits have therefore also been used. In these animals atherosclerosis can be readily and regularly produced by feeding cholesterol with or without fat. However, in the investigation of the influence of different dietary fats it appears less satisfactory to have to include cholesterol, which is capable by itself of influencing the lipid metabolism of the animal with hypercholesterolemia

and aortic changes as a consequence. It has generally been regarded as established that dietary fat does not *per se* cause any vessel changes, and feeding cholesterol has as yet been considered a *conditio sine qua non* for the induction of atherosclerosis in these animals.

The purpose of the present investigation was therefore to determine whether it might be possible to produce gross atherosclerotic changes in the aorta of rabbits by the use of a diet containing no supplementary cholesterol and not extremely rich in fat, whether dietary fats differ in their effect on the lipid partition in serum or liver of these animals, and whether such fats might have different atherogenic properties.

Chapter II

HISTORICAL

In 1904 MARCHAND suggested the term "atherosclerosis" for the type of human arteriosclerosis characterized by an accumulation of amorphous lipids in the intima. Until that time experimental attempts with adrenalin, digitalis, barium chloride, pathogenic bacteria and other agents had produced arterial lesions, but not atherosclerosis. The changes in the animals' vessels resembled rather the Mönckeberg type of arteriosclerosis in man (KATZ & STAMLER 1953).

Some years later IGNATOWSKI (1908) published his investigations on the effect of dietary animal protein such as meat, milk and eggs, on the metabolism and renal function in rabbits. IGNATOWSKI observed that some of the animals developed aortic changes resembling those seen in human atherosclerosis. These experiments, which were successfully reproduced by STAROKADOMSKY & SSOBOLEW (1909), who combined arterial hypertension with feeding eggs stirred in milk, and by FAHR (1912), prompted the hypothesis that the vessel changes were due to the injurious effect of the animal protein. Further experimentation with different kinds of foodstuffs revealed, however, that it was, above all, substances with a high fat content that had the strongest atherogenic effect (STUCKEY 1910). The most conspicuous aortic changes were observed when the rabbits were fed on egg yolk and brain substance, while no changes were observed when the animals were fed on the white of hens' eggs.

In the search for the active factor in egg yolk neither neutral fats (STUCKEY 1912) nor lecithin (WESSELKIN 1913) of itself was found to produce changes in the aortic wall. The nature of the active substance was not clarified until due consideration had been given to the investigations by CHALATOW (1911, 1912). This author had shown that feeding egg yolk or brain substance produced characteristic changes in the rabbit's liver and that these changes consisted of abundant infiltration of the liver parenchyma by fat-like substances, which in the fresh state assumed the form of birefringent droplets. Their physical properties — under polarized light the drops showed beautiful

cross figures — revealed them to be fluid sphero-crystals. According to ASCHOFF (1910), in the living organism these crystals consist almost exclusively of cholesterol-fatty acid esters.

Convincing experimental evidence in support of the conclusion that it was the cholesterol that was the atherogenic active factor in egg yolk was produced by ANITSCHKOW (1912), in collaboration with CHALATOW (1913), by their induction of typical atherosclerosis by feeding rabbits on pure cholesterol dissolved in sunflowerseed oil. After 1—4 months all of the rabbits showed intimal hyperplasia and birefringent fatty substances in the inner layer of the aortic wall. A supply of 25 g per day of sunflower oil without cholesterol produced no demonstrable changes. ANITSCHKOW therefore concluded "that the so called dietetic form of rabbit atherosclerosis is obviously due to the cholesterol or its compounds in the food" (ANITSCHKOW 1913, 1914).

Independently of these authors, WACKER & HUECK (1913) also found that inclusion of pure cholesterol in the ordinary diet was capable of producing aortic changes, which essentially resembled those seen in human atherosclerosis. The animals also developed hypercholesterolemia and pronounced cholesteatosis in the liver and adrenal cortex. While these investigations elucidated the important role played by exogenous cholesterol in the development of experimental atherosclerosis, also clinical investigators had, on the basis of chemical analysis of human material, suspected an intimate relationship between the cholesterol content of the blood and the atheromatous plaques in the aorta (LEMOINE 1911).

During the following years cholesterol feeding experiments were carried out by several investigators and owing to the positive results obtained such feeding has been regarded as the only reliable method for the experimental production of atherosclerosis. The negative results reported by a few authors (WELTMANN & BIACH 1913, VAN LEERSUM 1912, 1914, KNACK 1915) in rabbits fed cholesterol or a cholesterol-rich diet appear to be negligible compared with the numerous invariably positive results reported and confirming the findings first described by ANITSCHKOW and WACKER. (Readers interested in detailed surveys are referred to the comprehensive reviews by ANITSCHKOW 1933, LEARY 1936, HUEPER 1944, 1945, DUFF 1935, DUFF & MCMILLAN 1951, KATZ & STAMLER 1953, FRENCH 1955, KATZ et al. 1959).

In the above-mentioned investigations the animals received a diet containing cholesterol either in natural combination with the protein such as egg yolk, milk, brain substance, ground liver or meat, or — in most investigations — dissolved in vegetable oil such as sunflowerseed, olive, linseed, arachis- and cottonseed oil. In those experiments in which the animals

received these oils only, *i. e.* not in combination with cholesterol, the animals never developed atherosclerosis (ANITSCHKOW 1913, VERSÉ 1917, DUFF 1936).

Histochemical and chemical methods had previously shown an increased cholesterol content in human atherosclerotic plaques (VOGEL 1843, ASCHOFF 1907, WINDAUS 1910, SCHÖNHEIMER 1926, 1928). This together with the fact that it was possible to produce hypercholesterolemia and atherosclerosis in animals by cholesterol feeding and that there was obviously a correlation between hypercholesterolemia and atherosclerosis (DAVIS et al. 1937, ENGELBERG & NEWMAN 1943, FABER 1944, WILKINSON et al. 1948, ADLERSBERG et al. 1949) was long accepted as evidence that the cholesterol content of the food was of significance in the causation of hypercholesterolemia and atherosclerosis in man.

This opinion of the importance of exogenous cholesterol in the causation of atherosclerosis began to change after RITTENBERG & SCHÖNHEIMER (1937) had found evidence for the synthesis of cholesterol in mice from small units. The findings of these authors were confirmed by BLOCH & RITTENBERG (1942, 1945) in experiments using C^{13} and C^{14} indicating that acetate was the precursor substance of cholesterol. It could thus be regarded as established that the animal body itself could synthesize cholesterol, and since dietary fat, by oxidation of the fatty acid molecule, is broken down to acetate, which is then able to contribute to the synthesis of cholesterol, investigators began to focus interest on the total fat content of the diet. KEMPNER (1949), MELLINKOFF et al. (1950), WOLLAEGER et al. (1953) and KEYS (1953) showed that low-fat diets depressed the serum cholesterol content in patients with hypercholesterolemia. MALMROS (1950) and STRØM & JENSEN (1951) found a sharp fall in death-rate from circulatory diseases during World War II and showed that this drop was accompanied by a decrease in the consumption of milk, butter, cheese and eggs. That it was the dietary fat and not the cholesterol content of the diet that influenced the blood cholesterol level appeared probable in the light of the investigations by MESSINGER et al. (1950) and KEYS (1953), who produced evidence that the total serum cholesterol in man is independent of the amount of cholesterol normally ingested and that even a diet rich in cholesterol does not influence the serum cholesterol concentration.

In his book "Chinese Lessons to Western Medicine" SNAPPER (1941) suggested that the relatively low incidence of arteriosclerosis in China might have something to do with the fact that the Chinese diet includes abundant unsaturated fat, particularly in the form of peanut oil and sesame oil, which contain much linoleic acid. SNAPPER's suggestion was based on the investigations of BURR & BURR (1929), who had shown that certain fatty acids are essential for health and normal body growth. Rats on a diet completely free

of fat developed a typical deficiency syndrome characterized by weight loss, scaly skin, loss of hair, and skin sores, symptoms which could be controlled by inclusion in the diet of small amounts of the polyunsaturated acid linoleic acid, while saturated acids and even the mono-unsaturated oleic acid were ineffective in the treatment of this disease (BURR & BURR 1930).

In 1952 KINSELL et al. observed that in patients with various metabolic disorders a diet containing abundant vegetable fat depressed the cholesterol and phospholipid content of the blood, while an isocaloric substitution by animal fat resulted in a prompt increase of these lipids. At the same time GROEN et al. (1952) reported a 9 month ambulatory study on 60 normal human volunteers, in whom the total cholesterol content of the serum decreased gradually but significantly on replacement of the animal fat in the diet by vegetable fat. In a statistical investigation HARDINGE & STARE (1954) demonstrated that strict vegetarians usually had lower serum cholesterol levels than vegetarians who also consumed dairy products, and that in both groups the serum cholesterol level was lower than in the rest of the population.

These observations suggested that the nature of the dietary fat might be of greater significance for the lipid metabolism, and possibly also for atherogenesis, than the total amount of fat in the diet. In recent years this possibility has been studied systematically by KINSELL et al. (1953, 1954, 1955), COCHRANE et al. (1953), AHRENS et al. (1954, 1955, 1957), BEVERIDGE et al. (1955, 1956, 1957), FRISKEY et al. (1955), MALMROS & WIGAND (1955, 1957), BRONTE-STEWART et al. (1956), KEYS (1957 a, 1957 b), EGGSTEIN & SCHETTLER (1957), LEITNER (1957) and others.

These investigations confirmed the original findings by KINSELL and GROEN and showed that the consumption of vegetable oils with a high linoleic acid content such as safflower, sunflower, corn, cottonseed and peanut oil depresses the serum cholesterol level in normal persons and patients with hypercholesterolemia, while more saturated fats with a relatively small linoleic acid content such as butter, coconut oil, cocoa butter or palm oil produce no decrease but, if anything, a slight increase in the serum cholesterol. The changes in the cholesterol level were usually demonstrable already after 48 hours and, as a rule, reached a maximum after 2—3 weeks. Hydrogenation of oils deprived the latter of their ability to depress the serum cholesterol. That this difference in the effect of the fats is not due simply to their origin, *i. e.* animal or vegetable is apparent from the fact that certain vegetable oils such as cocoa fat and palm oil had the same effect as animal fat. It has also been shown that certain marine animal oils such as pilchard and whale oil have a depressive effect on the serum cholesterol

level, and that this effect disappears if these oils are hydrogenated (Bronte-Stewart et al. 1956, Malmros & Wigand 1957).

Recent experiments suggest that under certain circumstances dietary fats may have different effects on lipid metabolism and atherogenesis in rabbits. Thus Kritchevsky et al. (1954) in a study on the vehicle function of fat in cholesterol-fed rabbits showed that hypercholesterolemia and atheroma production was somewhat less pronounced when the fat consisted of corn oil than when it consisted of hydrogenated vegetable fat. While Kritchevsky et al. stated that the fat vehicles seemed to have no effect on the serum lipids or aorta atheroma of the rabbits when fed without cholesterol, Steiner & Dayton (1956) found an increase in the serum cholesterol content to several times its original value in rabbits fed on a diet consisting to 50—75 % of ground peanuts but not containing cholesterol. After 5—12 months 2 of 33 rabbits showed small areas of gross aortic atherosclerosis.

Recent papers by Lambert et al. (1958) and by Steiner et al. (1959), which were published while the present investigation was in progress (Wigand 1957) are commented upon in chapter VI.

Chapter III

MATERIAL

ANIMALS

Male rabbits of mixed stock were used. The animals were bought from local breeders and most of them were white. The rabbits were 7 months old at the beginning of the experiment and thus full-grown (BRAGDON 1952). On arrival at the department they were carefully examined for outer signs of disease and only apparently healthy animals were accepted for the experiment. During the entire experimental period the animals were kept in single hutches with a double bottom provided with perforations large enough to permit the passage of feces to prevent coprophagy. All the animals were housed indoors.

DIETS

For at least 1 month before commencement of the experiment proper the animals were allowed to eat green roughage and crushed barley *ad lib*. This was considered desirable in order to compensate any previous undernourishment.

The experimental basal diet was semi-synthetic. Its composition is given in Table 1. This is based on WOOLEY's (1954) diet No. 1436 for growth of rabbits. Deviations in the percentage of the ingredients were due to the necessity of including talcum and Aerosil (colloidal silicic acid) to facilitate preparation of the diet in tablet form. Corn starch was replaced by wheat starch. The cellulose content was increased somewhat to 19 % to secure feces of fairly normal size. Unless otherwise stated, the fat added to the basal diet represented 8.4 % by weight, which corresponded to 26 % of the calories. The lipid content of the basal diet was found to be 0.16 % (see Table 2). This fat was present mainly in casein, starch and cellulose. The lipid content of the basal diet was determined gravimetrically after reflux-boiling three times with ethanol-ethyl ether and once with acetone, re-extraction with chloroform-methanol, washing against water and subsequent re-extraction with petroleum ether and drying to constant weight in an exsiccator.

TABLE 1

Composition of experimental semi-synthetic diet

	% by weight	% of calories	*Salt mixture*	g/kg diet
Casein, purified [1]	25.26	34.4	$CaCO_3$	12.62
Saccharose	5.51	7.5	K_2HPO_4	7.58
Glucose	6.73	9.2	Na_2HPO_4	6.14
Wheat starch [2]	16.83	22.9	$Ca_3(PO_4)_2$	11.78
Fat [3]	8.42	26.0	NaCl	7.41
Cellulose [4]	18.94		$MgSO_4$ sicc.	2.78
Salt mixture	4.89		$FeSO_4$ sicc.	0.21
Choline chloride	0.25		$MnSO_4 \cdot 4H_2O$	0.35
Inositol	0.34		KJ	0.03
Ascorbic acid	0.42		$ZnCO_3$	0.02
p-Aminobenzoic acid	0.21		$CuSO_4 \cdot 5H_2O$	0.02
Potassium acetate	2.10			
Magnesium oxide	0.42		All salts were SP (Pharmacopoea Svecica, Ed. XI) or corresponding grade.	
Talc	3.37			
Aerosil [5]	6.31			
Other vitamins	mg/100 g			
A-palmitate	0.59			
Alpha-tocopherol acetate	1.96			
D_2	0.004			
K (Menadioli natril sulfas)	0.2		[1] Contained 0.21 % fat.	
Aneurine hydrochloride	1.59		[2] Contained 0.03 % fat.	
Pyridoxine hydrochloride	1.59		[3] See table 3, page 15.	
Riboflavin	1.59		[4] Made from saw-dust. Contained 0.08 % fat.	
Nicotinic acid	19.63			
Calcium pantothenate	3.93		[5] Colloidal silicic acid.	
Folic acid	0.59			
Biotin	0.059			
B_{12}	0.004			

The tablets weighed about 1 gram. They were made at Apoteket Hjorten, Lund, Sweden. After preparation the tablets were stored in sealed vessels at minus 20°C. Batches providing rations for about one week were taken from stock at a time.

The fats and oils used in the present experiments were: butter fat, hydrogenated coconut oil, corn oil, rapeseed oil, glyceryl trilaurate, glyceryl trilaurate plus linoleic acid and glyceryl trilaurate plus corn oil. The fatty acid composition and sterol content of these fats and oils are given in Table 2. The experimental diet was thus given without supplementary cholesterol. The

TABLE 2

Fatty acid composition of different diets and dietary fats used in the experiments

	Total lipid [1]	Fatty acids [2]			Sterol [3]
	% of dry matter	% saturated	% monounsaturated	% polyunsaturated	% of total lipid
Green roughage	3.19	24.4	11.8	63.8	1.81
Barley	2.58	30.4	13.4	56.2	2.09
Mangels	1.38	29.2	17.4	53.4	8.73
Semi-synthetic basal diet [4]	0.16	64.3	31.5	4.2	0.004
Butter fat [5]		58.6	38.0	3.4	0.27
Hydrogenated coconut oil, refined [6]		97.2 [7]	2.8	0	0.08
Corn oil, refined [6]		13.8	36.3	49.9 [8]	0.56
Rapeseed oil, refined [6]		11.6	64.9 [9]	23.5 [10]	0.38
Glyceryl trilaurate [6]		100.0 [11]	0	0	0
Linoleic acid [12]		0.9	9.2	89.9 [13]	0.03

[1] For details of the extraction procedure see page 12.

[2] The analyses were kindly performed by ROLF BLOMSTRAND, MD, Dept. of Clinical Chemistry, Univ. of Lund, by gas-liquid chromatographic separation mainly according to JAMES & MARTIN (1956).

[3] Determined by digitonin precipitation and Liebermann—Burchard reaction according to SPERRY & WEBB. (1950.)

[4] See table 1, page 13.

[5] Commercial product.

[6] A.B. Karlshamns Oljefabriker, Karlshamn, Sweden.

[7] 30 % lauric acid.

[8] 48.5 % linoleic acid.

[9] 50.3 % erucic acid.

[10] 14.8 % linoleic acid.

[11] 97.3% lauric acid.

[12] F. Hoffmann — La Roche & Co. AG, Basel, Switzerland.

[13] 87.9 % linoleic acid.

animals were allowed to drink tap water *ad lib*. The water was changed twice a day.

The control group received the above-mentioned diet consisting of green roughage and crushed barley except during the last few weeks, when the green roughage was out of season and was replaced by hay and mangels. The fat content and fatty acid composition of this control diet are given in Table 2.

TABLE 3

Experimental groups and experimental periods

Type of diet		Number of rabbits	Experimental period (weeks)
Basal diet	Added fat		
Semi-synthetic	8 % Butter fat	13	18
„	8 % Hydrogenated coconut oil	18	15
„	8 % Corn oil	16	15
„	8 % Rapeseed oil	15	18
„	8 % Glyceryl trilaurate	4	18
„	8 % Glyceryl trilaurate + 4 % Linoleic acid	2	18
„	8 % Glyceryl trilaurate + 4 % Corn oil	2	18
Green roughage + barley	—	9	18

EXPERIMENTAL GROUPS AND FEEDING PERIODS

The number of animals in the various experimental groups, the diets used and the feeding periods are given in Table 3. Originally the corn oil and coconut oil groups consisted of 20 rabbits each, but 3 animals in the former and 2 in the latter refused to eat the tablets and after 5 days, during which they had lost considerable weight, they were excluded from the experiment. On the 86th day of the experiment one rabbit in the corn oil group jumped out of its cage and developed paresis of the lower half of the body, bladder and intestines, and had to be killed. Autopsy revealed a fractured spine. This animal was not included in the analysis of the results either. This thus left 18 rabbits in the coconut oil group and 16 rabbits in the corn oil group.

The rapeseed oil and butter fat groups originally consisted of 15 rabbits each. In the butter fat group 2 rabbits were lost during the experiment. In 1 of the animals spinal fracture had occurred by accident when the animal was being weighed, while the other had to be killed because of spinal fracture of unknown origin. These 2 animals were excluded from the material. This thus left 15 rabbits in the rapeseed oil group and 13 rabbits in the butter fat group.

In another series a diet was tried that contained 8 % glyceryl trilaurate (4 rabbits) and the same diet with a supplement of 4 % linoleic acid or 4 % corn oil (2 rabbits each group). The smallnes of these groups was dictated by the high price of pure fatty acids.

The control group consisted of 9 animals.

The feeding period was 15 weeks for the coconut oil and corn oil groups and 18 weeks for the other groups. The shorter feeding for the coconut oil and corn oil groups was due to the fact that only a relatively small amount of tablets was available. The coconut oil and corn oil groups were studied during the summer of 1957; the other groups, during the summer of 1958.

Chapter IV

METHODS

BODY-WEIGHT, RATIONS, SAMPLING

The body-weight was determined twice a week throughout the feeding period. The animals were weighed in the morning before they had received their tablet ration.

The rations were calculated for each rabbit according to body-weight and adjusted twice a week. Since preliminary experiments had shown that 25 g tablets per kg body-weight seemed to be sufficient to cover the rabbits' daily requirements, this amount was chosen for the entire experimental period. The tablets were weighed for each rabbit every morning and placed in dishes, which were fastened to the wall of the cage. Any tablets or fragments spilt were collected twice a day and replaced in the dish. Tablets spilt during the night were collected and added to the next day's ration.

In order to be sure that the animals consumed an equal amount of tablets, the following procedure was adopted: The animals in the groups that were studied during the same period were matched for body-weight at the beginning of the experimental period. If a rabbit did not eat its entire tablet-ration, the following day's ration of its partner in the other group was diminished by a corresponding amount.

The analyses were carried out on blood samples collected from the marginal vein of the ear just before the commencement of the feeding period and once every three weeks throughout the experiment. The samples were collected in the morning, after the animals had fasted over night.

As a rule, about 10 ml blood was collected at a time. After the blood had clotted the samples were centrifuged and the serum was stored in small sealed test-tubes at minus 20—30°C until analyzed. Care was taken to prevent hemolysis, but if hemolysis did occur, a new sample was collected the following day.

The total serum cholesterol was determined on all serum samples, *i. e.* every third week. The lipid partition in serum was determined at the beginning and the end of the feeding periods. Serum lipoproteins as well as the fat excretion in the feces were determined at the end of the feeding periods.

The animals were killed by injection of 20—25 ml air into an ear vein. The liver was removed without delay and within 2—3 minutes a major portion of the specimen was placed in a refrigerator at minus 20—30°C until analyzed.

TOTAL CHOLESTEROL IN SERUM

The total serum cholesterol was determined by the method of ABELL et al. (1952) as modified by ANDERSON & KEYS (1956). 0.1 ml of serum free from hemolysis was hydrolyzed for 1 hour with alcoholic KOH at 37°C and then extracted 3 times with glass-distilled 40—60°C petroleum ether. After evaporation of the petroleum ether extract Liebermann-Burchard reagent was added under standardized temperature and the maximum optical density was determined after 30 and 35 minutes, respectively, at 610 mμ in a Beckman model B spectrophotometer using 10 mm cuvettes. Double determinations were made on each sample and every analytical series included a blank and double determinations on a standard solution of crystalline cholesterol (AB Kebo, Stockholm, Sweden). Cholesterol values were expressed as mg/100 ml.

The error of the method (S. D.) over the range from 1 to 1100 mg/100 ml was found to be ±1.94 %.

DETERMINATION OF LIPID PARTITION IN SERUM AND LIVER AFTER CHROMATOGRAPHIC FRACTIONATION ON SILICIC ACID COLUMN

EXTRACTION OF LIPIDS FROM SERUM

A suitable quantity of serum, as a rule 0.3—0.5 ml, with low lipid content also 0.7 ml, was extracted according to FOLCH et al. (1951), in 10 ml of a chloroform-methanol mixture 2: 1 by volume (the solvents were redistilled in all-glass apparatus before use). After filtering 2 suitable aliquots, as a rule 4 ml, were pipetted into glass tubes, which were then carefully filled with distilled water, after which the tubes were placed in a beaker with distilled water of at least 10-fold the volume of extract to be washed. After standing overnight (at least 16 hours) the water phase was removed first by suction and then by pipette as completely as possible, the remaining "fluff" being brought to solution by a few drops of methanol. Under these conditions extraction of lipids is quantitative (SPERRY 1955).

The clear, washed extract was evaporated on a water bath under a continuous stream of nitrogen and re-extracted immediately with about 0.5 ml petroleum ether (distilled in all-glass apparatus, b. p. 40—60°C). The lipid solution was then transferred quantitatively to a silicic acid column.

EXTRACTION OF LIPIDS FROM LIVER

In order to permit use of the same chromatographic system as for the serum lipid analyses, relatively small samples of liver were analyzed in the determination of the lipid partition.

Since determination of the fresh and dry weight of small tissue samples involves serious sources of error, *inter alia* because of rapid evaporation of moisture from the pieces of tissue (BILLING et al. 1953), it was considered advisable, after lipid extraction, to determine the content of nitrogen in the liver specimen and calculate the concentration lipids per mg nitrogen. With the method used homogenization, extraction and determination of the nitrogen content could be performed in one and the same tube. This eliminated the sources of error involved by the transport of substance from and to different containers.

Suitable samples of about 50—100 mg wet weight were removed directly from the frozen liver, care being taken to avoid the capsule and the great vessels and transferred immediately to micro-tubes of Pyrex glass (length: 120 mm, diameter: 9 mm, material: 1 mm.). Chloroform-methanol mixture 2: 1 by volume was added and the tissue specimen homogenized within 15 minutes with the aid of a pestle using a motor of 1/6 HP and 1,425 RPM (BERGSTRÖM & GLOOR 1954). Homogenization required about 30—60 sec. Microscopic inspection showed the homogenate obtained in this way to contain hardly any intact cells.

The homogenate was extracted with 3 successive portions of chloroform-methanol, total about 12—15 ml, the tube being centrifuged before removing the supernatant. The combined extract was collected in a 10 ml volumetric flask the excess solvent being evaporated under a stream of nitrogen. Two 4 ml aliquots (2 ml if lipid content was high) were pipetted into suitable glass tubes and washed with distilled water in the way described in the foregoing section.

FRACTIONATION PROCEDURE

The chromatographic fractionation of serum and liver lipids on silicic acid column was based on the method of BORGSTRÖM (1952 a, 1952 b), eluation of the fractions was done essentially according to the method of FILLERUP & MEAD (1953) and MEAD & FILLERUP (1954) as described by GARTON & DUNCAN (1957) except for the elution of the phospholipid fraction for which pure methanol was used (CARLSON & WADSTRÖM 1958).

Silicic acid column: — Ordinary 20 ml volumetric pipettes with an inner diameter of 5 mm at the lower end were used as glass tubes. Silicic acid powder ("Baker Analyzed" reagent) and Celite Analytical Filter Aid (Johns Manville) were thoroughly washed with hot methanol (redistilled in all-glass

apparatus), warm peroxide-free ether (redistilled and stored over KOH) and acetone (redistilled in all-glass apparatus), dried and activated at 120°C for 24 hours and stored in well-filled, air-tight glass vessels for at most one week. For each fractionation 200 mg silicic acid and 200 mg Celite were thoroughly mixed and transferred into the 20 ml pipette filled to the brim with petroleum ether (redistilled in all-glass apparatus, b. p. 40—60°C), in which they readily settled without sticking to the walls. The mixture was packed in the lower end of the pipette under light pressure from a nitrogen gas tube to give a column of about 5 mm×60 mm, supported on a thin layer of sand (pro analysi, washed in acid and glowed, AB Kebo, Stockholm, Sweden) and a small layer of cotton wool. About 10 ml of petroleum ether was allowed to run through the column, after which the rest was removed by gentle suction.

The lipid extract (see page 18) was transferred quantitatively onto the column with the aid of a long capillary pipette.

Elution. — Elution was started as soon as the petroleum ether solution had entered the column. Sterol esters, triglycerides and free sterols were removed with 12 ml of 1 %, 3 % and 10 % ethyl ether in petroleum ether, respectively. The phospholipid fraction was eluted with 25 ml pure methanol. A rate of elution of about 15—18 drops per minute was secured by gentle pressure from a nitrogen gas tube.

Double determinations were made on each sample, *i. e.* fractionation on two columns. A blank column and two columns with a standard triglyceride solution were eluted at the same time as the samples.

In order to check the efficiency of the fractionation a mixture of cholesterol, cholesterol esters, triglycerides and phospholipids obtained by chromatographic purification of a lipid extract from serum was eluted. The concentrations of the four lipids were determined chemically by the methods described above both before and after elution from the silicic acid column. In addition, the cholesterol, triglyceride and phospholipid content was determined in each fraction. The results showed that with the elution quantities given above separation of the 4 fractions was satisfactory.

The recovery was found to be 101 % for cholesterol, 94 % for cholesterol-ester, 98 % for triglycerides and 95 % for phospholipids.

As a further check a serum of known composition was run through the whole procedure from time to time.

DETERMINATION OF FREE AND ESTER-BOUND CHOLESTEROL

Both cholesterol fractions were collected in 110×15 mm glass tubes with ground glass stoppers. The eluate was evaporated to dryness under a conti-

nuous stream of nitrogen and hydrolyzed for 1 hour at 37°C with 2 ml alcoholic KOH. The cholesterol content was then determined by the method given on page 18. The values were expressed as mg/100 ml (for serum) or μg/mg nitrogen (for liver).

The error of the method (S. D.) for free cholesterol in serum over the range from 1 to 600 mg/100 ml was found to be ± 4.22 %, for free cholesterol in liver (20 double determinations) over the range from 1 to 200 μg/mg nitrogen ± 9.63 %, for ester-bound cholesterol in serum over the range from 1 to 800 mg/100 ml ± 4.27 %, and for ester-bound cholesterol in liver over the range from 1 to 700 μg/mg nitrogen ± 6.83 %. The error of the method includes all possible sources of error influencing the method from the beginning of the procedure, *i. e.* during lipid extraction, during elution from the silicic acid column and during the actual determination of cholesterol.

The total cholesterol was calculated as the sum of free and ester-bound cholesterol. As this figure resembles a sum, no statistical analysis of the error of the method is given. A comparison, based on the data from the present investigation, of these values for total cholesterol with those obtained by the method of ABELL shows that they were about 7 % lower presumably owing to a somewhat incomplete recovery of cholesterol esters from the silicic acid column.

DETERMINATION OF TRIGLYCERIDES

The triglyceride fraction was collected in 110×15 mm glass tubes with ground glass stoppers. The eluate was evaporated to dryness under a continuous stream of nitrogen and redissolved in 1 ml ethanol (95 %, AB Svenska Vin- och Spritcentralen). The triglyceride content was then determined according to the method of LAMBERT & NEISH (1950), as modified by CARLSON & WADSTRÖM (1959). This method is based principally upon the determination of the glyceride glycerol, which is quantitatively oxidized to formaldehyde by periodic acid and then determined by the colour reaction with chromotropic acid. One blank and two standard solutions (Triolein, Merck A. G.) were run simultaneously through the whole procedure.

The optical density was determined at 570 mμ in a Beckman model B spectrophotometer using 10 mm cuvettes. If the extinction value exceeded 1.0, oxidation was repeated with a smaller amount of the sulphuric acid solution The triglyceride values were calculated as glyceryl trioleate.

The error of the method (S. D.) for triglycerides in serum over the range from 1 to 1300 mg/100 ml was found to be ± 5.01 % and for triglycerides in liver, over the range from 1 to 1500 μg/mg nitrogen ± 6.71 %.

The error of the method includes all possible sources of error influencing

the method from the beginning of the procedure, *i. e.* during lipid extraction, during elution from the silicic acid column and during the actual analysis of the glycerides.

DETERMINATION OF PHOSPHOLIPIDS

The phospholipid fraction was collected in 180×18 mm Pyrex glass tubes. The eluate was evaporated to dryness under a continuous stream of nitrogen. The phosphorus content was then determined by the method of AMMON & HINSBERG (1936), as modified by CHEN et al. (1956). The dried phospholipid extract was ashed with concentrated sulphuric acid and the colour formed by the reduction of phosphomolybdate by ascorbic acid was determined. One blank and double standard solutions of KH_2PO_4 were run through the procedure simultaneously. The optical density was determined at 820 mμ in a Beckman model B spectrophotometer using 10 mm cuvettes. The factor 25 was used for conversion from phosphorus to phospholipids.

The error of the method (S. D.) for phospholipids in serum over the range from 1 to 1100 mg/100 ml was found to be ±4.14 % and for phospholipids in liver (40 double determinations) , over the range from 600 to 1400 μg/mg nitrogen ±4.23 %.

The error of the method includes all possible sources of error influencing the method from the beginning of the procedure, *i. e.* during lipid extraction, during elution from the silicic acid column and during the actual determination of the phosphorus.

DETERMINATION OF TOTAL LIPIDS

Total lipids in serum and liver were calculated as the sum of free cholesterol + cholesteryl esters + triglycerides + phospholipids, the cholesteryl esters being calculated as oleate by multiplying the values for ester-bound cholesterol by 1.68. As this figure resembles a sum, no statistical analysis of the error of the method is given.

DETERMINATION OF NITROGEN CONTENT IN LIVER

After extraction with chloroform-methanol (see page 19) the nitrogen content of the liver homogenate was determined according to the method of MILLER & HOUGHTON (1945), which is based on the Kjeldahl method for determination of nitrogen. The analyses were carried out at the Clinical Laboratory, University Hospital, Lund, with the technic and the apparatus used there. After combustion of the protein sample with sulphuric acid and potassium sulphate and with mercuric oxide as a catalyst the sample was made alcaline and thiosulphate was added. The alcaline sample was then

stem-distilled in all-glass apparatus. The ammonia released was collected in a known amount of sulphuric acid, and the excess acid was titrated with NaOH.

In order to test the accuracy of the distillation procedure, the recovery of nitrogen from a standard solution of pure dried ammonium sulphate was determined. 10 aliquots containing 0.6720 mg nitrogen each were used and the average recovery found to be 0.6695 mg, which is equivalent to 0.372 % below the theoretical.

DETERMINATION OF LIPID PARTITION IN DIFFERENT PARTS OF THE LIVER

The equal distribution of the different lipid fractions throughout the liver was checked by determining the lipid partition in 6 specimens obtained from each of the livers from 6 rabbits. The liver samples were taken from different parts of the lobus sinister, lobus sinister accessorius and processus caudatus and the lipid fractions determined according to the methods described above. The data obtained from 3 livers with different lipid contents are summarized in Table 4, which shows that the distribution of the different lipids throughout the liver was uniform. A similar uniform distribution was also found in the other 3 livers studied.

DETERMINATION OF LIPOPROTEINS IN SERUM

The lipoproteins in the serum were determined after electrophoretical separation on filter paper using the apparatus and the buffer described by Laurell et al. (1956). The lipids were stained with Sudan Black according to the method of Swahn (1952, 1953), as was the eluation of the colour. The optical density was read at 590 mμ in a Beckman model B spectrophotometer using 10 mm cuvettes.

Owing to the difficulty in making an exact distinction between the β-fraction and the starting fraction on the paper, the values are given as α- and $\beta+0$-lipoproteins.

The error of the method (S. D.) (40 double determinations) was found to be ± 9.91 % for the α-fraction and ± 3.17 % for the $\beta+0$-fraction.

DETERMINATION OF FAT EXCRETION IN FECES

The feces were collected towards the end of the experiment on 5 consecutive days and stored in 95 % ethanol until analyzed.

The total fatty acids and the non-saponifiable fraction in the feces were

TABLE 4

Lipid partition in different parts of rabbit liver[1] *(μg/mg N)*

Liver No.	Sample No.	Free cholesterol	Ester-bound cholesterol	Total cholesterol	Tri-glycerides[2]	Phospho-lipids[3]	Total lipids[4]
1	1	104	56	160	131	728	1057
	2	102	58	160	93	730	1022
	3	113	62	175	123	778	1118
	4	104	58	162	112	763	1076
	5	107	60	167	113	760	1081
	6	107	54	161	95	745	1038
	Mean:	106	58	164	111	751	1065
2	1	96	39	135	396	995	1553
	2	98	38	136	373	985	1520
	3	92	44	136	402	950	1518
	4	101	39	140	430	1040	1637
	5	87	36	123	355	1025	1527
	6	92	38	130	398	970	1524
	Mean:	94	39	133	392	994	1547
3	1	123	304	427	820	813	2266
	2	122	339	461	770	844	2306
	3	126	320	446	780	918	2362
	4	127	348	475	865	823	2400
	5	125	322	447	795	867	2328
	6	125	312	437	880	866	2395
	Mean:	125	324	449	818	855	2343

[1] See page 23.

[2] Calculated as glyceryl trioleate.

[3] Lipid phosphorus × 25.

[4] Free cholesterol + cholesteryl esters + triglycerides + phospholipids. Cholesteryl esters were calculated as cholesteryl oleate by multiplying the values for ester-bound cholesterol by 1.68.

determined according to the technique described by BLOMSTRAND & LINDQUIST (1955), which is based on the method of VAN DE KAMER et al. (1949).

The technique was briefly as follows. After homogenization in a Starmix blender the feces were refluxed several times with ethanol-ethyl ether and ether alone followed by re-extraction of the combined filtrate with ethyl-ether. After saponification with KOH the non-saponifiable fraction was removed by repeated extraction with petroleum ether. After acidifying with HCl the fatty acids were extracted with petroleum ether. Both fractions were

collected separately and, after evaporating the petroleum ether under nitrogen, dried to constant weight.

In order to check the accuracy of the method double determinations were carried out on 5 feces samples. The following values were found (mg):

	Non-saponifiable fraction	Total fatty acids
1.	86.85	200.75
	88.60	249.20
2.	139.25	337.20
	132.78	360.48
3.	143.50	213.15
	138.18	233.95
4.	94.30	372.00
	81.48	374.13
5.	51.75	755.35
	46.18	734.53

The absorption percentage of the different fats fed was calculated according to the following formula:

$$\frac{\text{g ingested fatty acids} - \text{g excreted fatty acids}}{\text{g ingested fatty acids}} \times 100$$

GRADING OF AORTIC CHANGES

The entire aorta was removed from the heart valves down to the bifurcation, dissected free from contiguous fat and connective tissue, and fixed in 10 % alcaline formalin. At the latest within a few days the gross changes on the intimal surface were judged after staining with Sudan IV in 70 % ethanol-acetone, 1: 1 by volume. This stain turns lipid deposits red and was used in order to facilitate the detection of even small changes.

The gross changes were judged by two persons independently of one another and without knowledge of the group to which the animal belonged. The following grades were recognized:

0 — normal appearance

\+ — single small spots or streaks predominantly in ascending and thoracic aorta.

\+\+ — more abundant, larger, often confluent plaques in ascending, thoracic and abdominal aorta and the heart valves.

\+\+\+ — larger regions of entire aorta covered with raised, confluent plaques.

STATISTICAL METHODS

In the statistical treatment of the data the following procedures described by DAVIES (1949) were adopted.

The following formulae were used:

$$\text{The mean value } \bar{x} = \frac{\Sigma x}{n}$$

where x denotes single observations and n the number of observations.

The standard error of the mean:

$$\text{S. E. } (\bar{x}) = \sqrt{\frac{\Sigma (x - \bar{x})^2}{n(n-1)}}$$

To determine whether the difference between two mean values, $\bar{x}_1$ and $\bar{x}_2$, was significant, the number of observations being n_1 and n_2 respectively, the t-test was used:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\text{S. E. } (\bar{x}_1 - \bar{x}_2)}$$

where the standard error of the difference:

$$\text{S. E. } (\bar{x}_1 - \bar{x}_2) = \sqrt{\frac{\Sigma (x_1 - \bar{x}_1)^2 + \Sigma (x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2} \cdot \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

In the treatment of pairs of observations, *i. e.* one at the beginning and one at the end of experiment, the difference between the two values was analyzed according to the difference method:

$$\bar{d} = \frac{\Sigma d}{n}$$

where d denotes the difference between the beginning and the end values in the successive pairs and n the number of pairs.

The t-value was then calculated according to the following formula:

$$t = \frac{\bar{d}}{\text{S. E. } (d)}$$

$$\text{S. E. } (\bar{d}) = \sqrt{\frac{\Sigma (d - \bar{d})^2}{n(n-1)}}$$

When p was less than 0.05, the differences were regarded as significant.

The correlation coefficient r between two variates x and y was calculated according to the following formula:

$$r = \frac{\Sigma (x - \bar{x}) \cdot (y - \bar{y})}{\sqrt{\Sigma (x - \bar{x})^2 \cdot \Sigma (y - \bar{y})^2}}$$

The error of the method (=standard error of a single determination=S.D.) was studied according to DAHLBERG (1948) by statistical analysis of the values obtained, unless otherwise stated, from 60 double determinations (i. e. double test performed on different serum or liver samples). The following formula was used:

$$\text{S. D.} = \sqrt{\frac{\Sigma\, d^2}{2\,n}}$$

where d denotes the difference between the two single tests performed on each sample and n the number of double determinations. The values for S. D. were expressed as per cent.

Chapter V

RESULTS

GENERAL OBSERVATIONS

When tablet feeding was started, the animals usually required 1—3 days to get accustomed to the new diet. Most of the animals soon learnt how to eat the tablets sitting close to the dish without spilling any of the tablets. The tablets appeared to be palatable, the rabbits usually eating up their ration in the course of the day with the exception that the groups on hydrogenated coconut oil or trilaurate did not finish off their ration until later in the evening.

The diet consumption was as a rule 25 g tablets per kg body-weight per day in all groups. Only occasionally did an animal not eat up its entire ration, and then usually only in the coconut oil group. Since the feeding schema described above was applied throughout, groups studied simultaneously consumed an equal amount, also during these periods.

The semi-synthetic diet produced a change in the appearance and consistence of the feces, the "currants" becoming fairly pale and slightly smaller than those observed in the control group fed on green roughage and barley. In none of the groups was any persistent diarrhea observed.

No signs of vitamin deficiency such as photophobia, conjunctivitis, convulsions, paresis or haemorrhages were observed. All animals except those referred to on page 15 which had to be killed because of an accident, completed the experimental periods of 15 and 18 weeks, respectively. That rabbits can be kept on this diet also for longer periods is apparent from a small series of 5 animals, which received the tablet diet for a period of 54 weeks.

BODY-WEIGHT

The body-weights noted in the various groups during the experimental periods are given in Table 5 and Figs. 1 and 2. Only values recorded at the beginning of the experiment and at 7.5 and 15 weeks were treated statistically. In all of the groups the average body-weight of the animals was between 2,600 and 3,000 g when tablet feeding was started. The control group

TABLE 5

Body weight (g) of rabbits fed different dietary fats

Groups	0 week	7.5 weeks		15 weeks	
	Mean ± S. E.	Mean ± S. E.	Diff.[1] ± S. E.	Mean ± S. E.	Diff.[1] ± S. E.
Controls	2659.0±154.0	2802.0±172.0	143.3±54.9 $p < 0.05$	2802.0±157.0	143.3±45.2 $p < 0.025$
Butter fat	2862.0±102.0	3080.0±165.0	217.7±70.9 $p < 0.01$	3200.0±168.0	337.7±83.4 $p < 0.005$
Hydrogenated coconut oil	2993.0±79.0	2961.0±118.0	—32.2±69.0 $p > 0.05$	3052.0±127.0	58.9±71.7 $p > 0.05$
Corn oil	2860.0±84.0	3149.0±138.0	289.4±81.4 $p < 0.005$	3331.0±114.0	470.6±59.2 $p < 0.001$
Rapeseed oil	2817.0±85.0	3016.0±104.0	199.3±36.2 $p < 0.001$	3221.0±116.0	404.7±53.8 $p < 0.001$
Glyceryl trilaurate	2605.0±167.0	2578.0±108.0	—27.5±189.0	2440.0±83.0	-165.0±236.0
Glyceryl trilaurate + linoleic acid	2810.0±90.0	3040.0±20.0	230.0±70.0	3100.0±10.0	290.0±80.0
Glyceryl trilaurate + corn oil	2700.0±40.0	3095.0±235.0	395.0±195.0	3380.0±410.0	680.0±370.0

[1] Calculated from 0 week.

fed green roughage and barley showed a continuous moderate increase of 140 g during the first 7.5 weeks, after which body-weight remained constant.

In the butter fat, corn oil and rapeseed oil groups a continuous increase in weight was noted throughout the feeding period in all animals. After 15 weeks the body-weight in these groups was about 300—500 g higher than at the beginning of the experiment. The increases were statistically significant.

In the group fed hydrogenated coconut oil there was a tendency to loss of body-weight after 7.5 weeks and after 15 weeks body-weight did not differ significantly from that at the beginning of the experiment. Five of the animals in this group showed a successive loss in weight of 90 to 640 g.

A reduction in body-weight was also noted for 3 of the 4 rabbits that received 8 % glyceryl trilaurate in the diet, while those that received 4 % linoleic acid or 4 % corn oil in addition to the trilaurate increased successively in weight. Owing to the smallness of these three last-mentioned groups the data were not treated statistically.

Compared with the control group, after 15 weeks on tablet diet both the

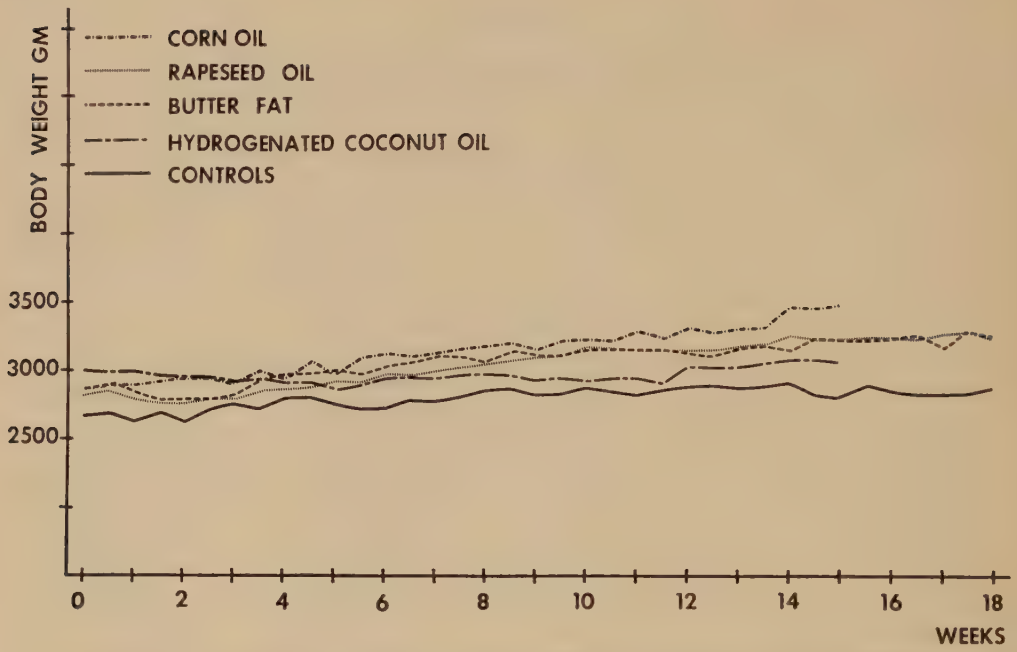

Fig. 1. Body-weight of rabbits fed 8 % different dietary fats. The controls were fed on green roughage and barley.

TABLE 6. *Total serum cholesterol levels* [1] *in rabbit*

Groups	0 week	3 weeks		6 weeks	
	Mean ± S. E.	Mean ± S. E.	Diff. [2] ± S. E.	Mean ± S. E.	Diff. [2] ± S. E.
Controls	25.3±2.4	28.9±4.0	3.6±2.9 $p > 0.05$	30.9±2.6	5.6±3.7 $p > 0.05$
Butter fat	28.7±1.9	93.2±11.8	64.5±10.5 $p < 0.001$	177.7±29.0	149.8±28.2 $p < 0.001$
Hydrogenated coconut oil	32.2±2.2	82.8±9.3	50.6±9.1 $p < 0.001$	177.9±14.7	145.8±14.5 $p < 0.001$
Corn oil	29.8±1.9	44.6±4.0	14.9±3.5 $p < 0.001$	67.9±12.0	38.1±11.6 $p < 0.005$
Rapeseed oil	30.8±1.6	42.7±2.9	11.9±3.2 $p < 0.005$	63.6±3.9	32.8±3.3 $p < 0.001$
Glyceryl trilaurate	34.0±3.7	61.5±17.4	27.5±15.6	147.8±29.4	113.8±30.0
Glyceryl trilaurate + linoleic acid	34.5±3.5	58.0±4.0	23.5±7.5	104.5±16.5	70.0±13.0
Glyceryl trilaurate + corn oil	57.5±25.5	51.0±23.0	—6.5±2.5	69.0±29.0	11.5±3.5

[1] ABELL *et al.* (1952). [2] Calculated from 0 week.

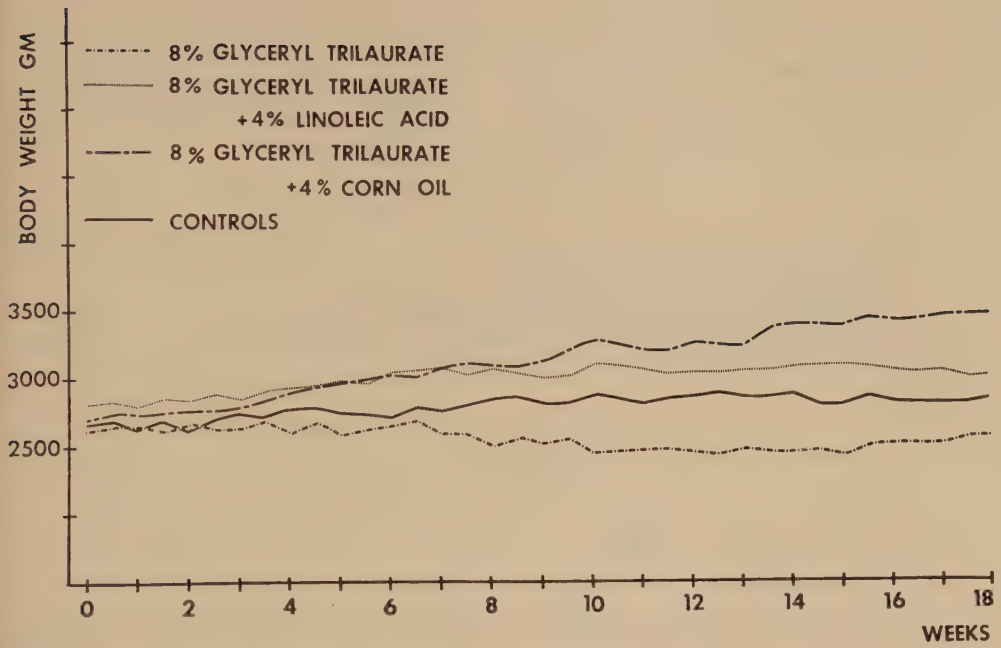

Fig. 2. Body-weight of rabbits fed 8 % glyceryl trilaurate with and without addition of 4 % linoleic acid or corn oil. The controls were fed on green roughage and barley.

ed different dietary fats (mg/100 ml)

9 weeks		12 weeks		15 weeks		18 weeks	
Mean ± S. E.	Diff. [2] ± S. E.	Mean ± S. E.	Diff. [2] ± S. E.	Mean ± S. E.	Diff. [2] ± S. E.	Mean ± S. E.	Diff. [2] ± S. E.
29.4±2.3	4.1±2.1 $p > 0.05$	32.1±2.9	6.8±2.6 $p < 0.05$	29.6±3.0	4.2±4.1 $p > 0.05$	22.6±2.8	—2.8±2.3 $p > 0.05$
271.7±28.2	243.0±37.9 $p < 0.001$	290.4±37.6	261.7±37.7 $p < 0.001$	312.1±44.7	283.4±44.6 $p > 0.001$	357.5±49.5	328.8±49.1 $p < 0.001$
321.2±41.0	289.0±41.0 $p < 0.001$	414.6±50.8	382.4±50.6 $p < 0.001$	557.6±81.3	525.4±80.8 $p < 0.001$	—	—
74.3±10.7	44.5±10.4 $p < 0.001$	65.9±10.9	36.1±10.4 $p < 0.005$	69.3±11.6	39.5±11.3 $p < 0.005$	—	—
60.1±5.2	29.3±5.0 $p < 0.001$	66.0±7.2	35.2±7.3 $p < 0.001$	76.3±7.6	45.5±7.4 $p < 0.001$	71.5±12.0	40.7±11.7 $p < 0.005$
13.5±106.3	379.5±109.7	765.3±144.7	731.3±148.4	966.5±137.6	932.5±139.1	664.8±79.6	630.8±83.3
206.0±0	171.5±3.5	224.0±33.0	189.5±29.5	346.5±101.5	312.0±98.0	449.0±86.0	414.5±82.5
154.0±51.0	96.5±25.5	160.5±74.5	103.0±49.0	191.0±77.0	133.5±51.5	141.0±64.0	83.5±38.5

corn oil group and the rapeseed oil group showed a significantly higher body-weight ($p<0.001$ and <0.025, respectively), while the difference between the butter fat or coconut oil fed animals on the one hand and the controls on the other, was not statistically significant ($p>0.05$).

Summary

Feeding adult rabbits on butter fat, corn oil or rapeseed oil at the 8 % level in a cholesterol-free semi-synthetic diet resulted in a moderate increase in body-weight, while animals fed hydrogenated coconut oil or glyceryl trilaurate showed no increase in weight, but, if anything, a tendency to weight loss.

TOTAL CHOLESTEROL IN SERUM

The average values found for the total serum cholesterol levels in the different groups are given in Table 6.

Before the beginning of the experiment proper, when the animals were on an ordinary diet consisting of green roughage and barley, the serum cholesterol was low; in all groups it lay between 25 and 58 mg/100 ml. In the control group, which continued to receive its ordinary diet, the cholesterol level persisted essentially unchanged during the 18 weeks the experiment lasted (Fig. 3), the values after 15 and 18 weeks not being significantly different from the starting value.

In both the rapeseed oil and the corn oil groups the average serum cholesterol showed a slight increase, mainly during the first weeks, to remain fairly steady afterwards (Fig. 8). At the end of the experiment the serum cholesterol level was 69 mg/100 ml for the corn oil and 72 mg/100 ml for the

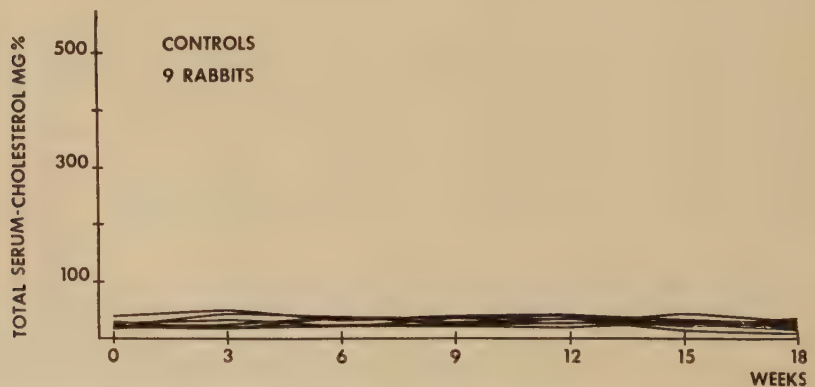

Fig. 3. Total serum cholesterol in control rabbits fed on green roughage and barley.

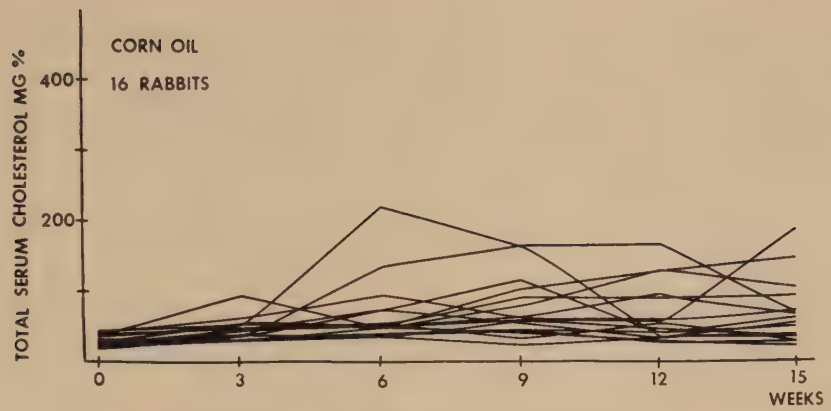

Fig. 4. Total serum cholesterol in rabbits fed 8 % corn oil.

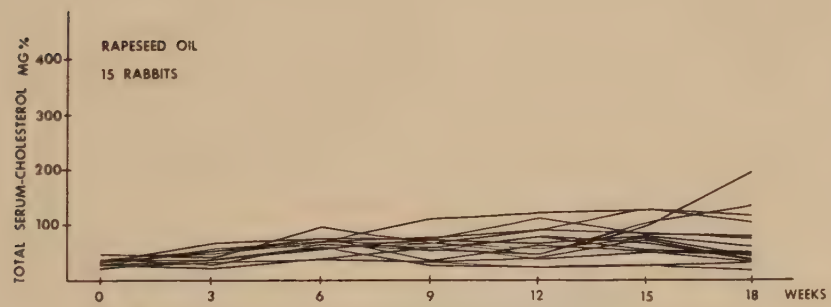

Fig. 5. Total serum cholesterol in rabbits fed 8 % rapeseed oil.

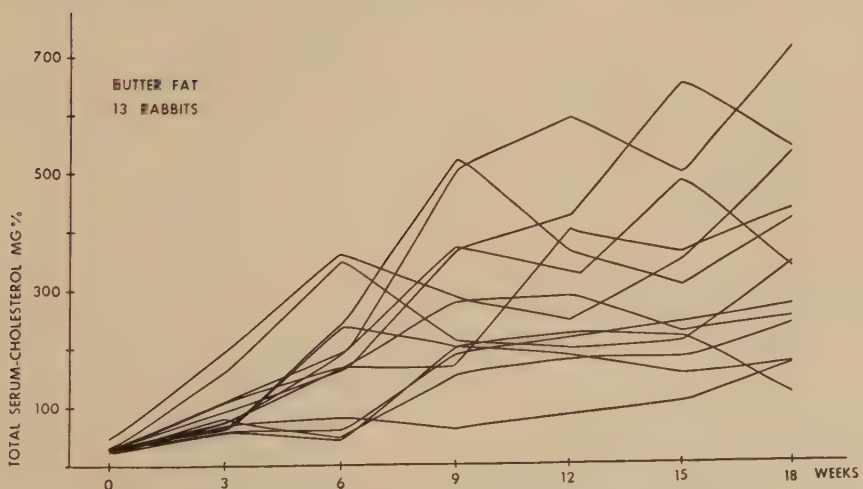

Fig.6. Total serum cholesterol in rabbits fed 8 % butter fat.

rapeseed oil group, this increase being statistically significant for both groups. The individual values are given in Figs. 4 and 5. Two rabbits in the corn oil group showed a transient increase in the serum cholesterol to above 150 mg/100 ml. In 2 other animals values of 150 and 190 mg/100 ml were observed at the end of the experiment. One rabbit in the rapeseed oil group had a final serum cholesterol level of 200 mg/100 ml; otherwise the serum cholesterol values in this group never exceeded 140 mg/100 ml.

Comparison at 15 weeks between these two groups and the control group showed that the serum cholesterol, though only slightly increased, was significantly higher in the corn oil group ($p<0.05$) as well as in the rapeseed oil group ($p<0.001$). No difference was found between the two oil-fed groups ($p>0.05$).

In the butter fat group the serum cholesterol increased on the average from 29 to 358 mg/100 ml. This increase was statistically significant at the 0.1 % level. As is apparent from Fig. 6, in most of the animals the cholesterol began to rise between the third and sixth week and at the end of the experiment, after 18 weeks, the values were, with but one exception, higher than 150 mg/100 ml, the highest value observed in this group being 733 mg/100 ml. It is also clear from the figure that the cholesterol values fluctuated considerably in each animal as well as from one animal to another. One rabbit in this group showed some kind of "resistance" and had, as the only animal in the group, as late as 12 weeks after the beginning of the feeding period a cholesterol value of less than 100 mg/100 ml. Not until afterwards did the cholesterol level rise to 173 mg/100 ml. The mean values for this group (Fig. 8) showed a continuous increase in the serum cholesterol throughout the experimental period.

As calculated from the values at 15 weeks after the beginning of the feeding period, the hypercholesterolemic response in the butter fat fed animals was significant at the 0.1 % level when compared with the control group or the groups receiving corn oil and rapeseed oil, respectively.

A hypercholesterolemia still more marked than that observed in the butter fat group was noted in the animals that received 8 % hydrogenated coconut oil in the diet (Fig. 7). In this group the serum cholesterol also began to rise in almost all animals between the third and sixth week after the beginning of the experiment and then continued to do so throughout the experimental period to an average value of 558 mg/100 ml. This hypercholesterolemic response was statistically significant at the 0.1 % level. In three animals values higher than 1000 mg/100 ml were noted, the highest value being 1420 mg/100 ml. The serum cholesterol levels for the individual animals showed a fairly wide fluctuation during the entire experimental period. In this group,

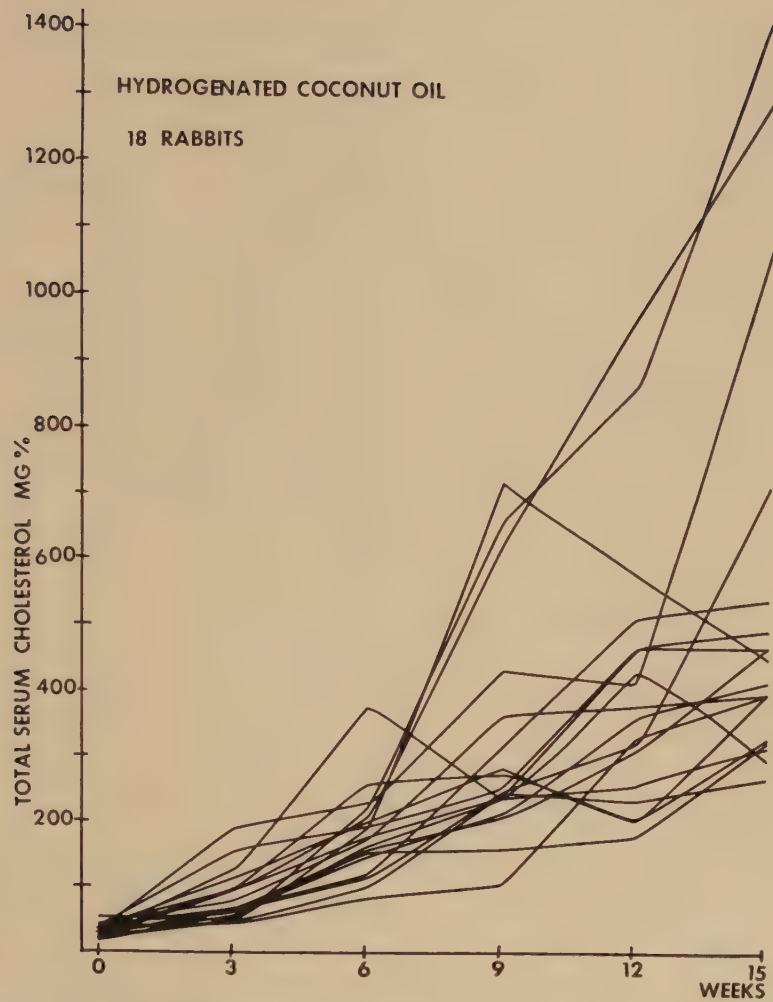

Fig. 7. Total serum cholesterol in rabbits fed 8 % hydrogenated coconut oil.

as in the group fed butter fat, there was one rabbit in which the serum cholesterol remained fairly low for a long time. Not until the 12th week did this animal show definite hypercholesterolemia.

On comparison with the control group fed green roughage and barley the hypercholesterolemia observed in the coconut oil fed group was statistically significant at the 0.1 % level as was the difference between this group and those fed corn oil or rapeseed oil. The coconut oil fed animals also showed a significantly more pronounced hypercholesterolemic response than those

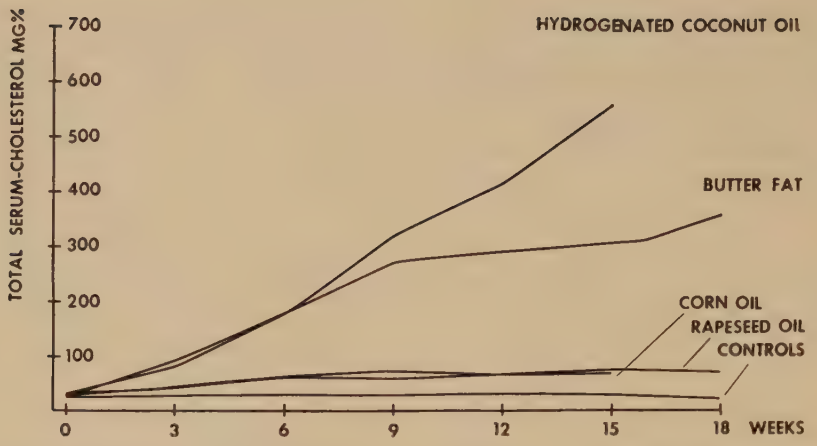

Fig. 8. Average total serum cholesterol in rabbits fed 8 % different dietary fats. The controls were fed on green roughage and barley.

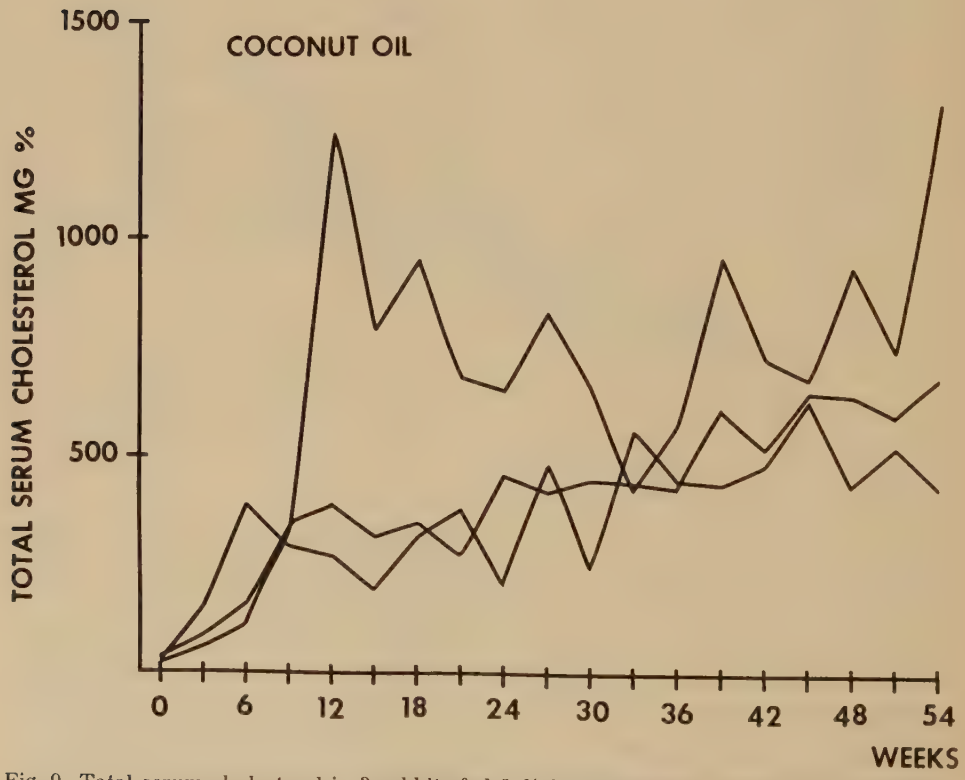

Fig. 9. Total serum cholesterol in 3 rabbits fed 8 % hydrogenated coconut oil for 54 weeks.

Fig. 10. Total serum cholesterol in 2 rabbits fed 8 % hydrogenated coconut oil for 18 weeks and 8 % corn oil for subsequent 33 weeks.

fed butter fat ($p<0.025$). The curves for the mean values in the various groups are given in Fig. 8.

To be sure that the hypercholesterolemic effect of hydrogenated coconut oil was not of transient nature, 3 rabbits were fed 8 % hydrogenated coconut oil for 54 weeks. The serum cholesterol began to rise distinctly in all 3 animals after the third to sixth week and then in one of the animals it rose sharply to 1237 mg/100 ml, after which it fell again to a lower level (Fig. 9). Despite a fairly wide fluctuation, on the whole, the cholesterol values showed

a continuous rise to reach 435, 678 and 1310 mg/100 ml, respectively within 54 weeks. These results indicate that the hypercholesterolemia induced by feeding hydrogenated coconut oil is not of transient nature but persists as long as coconut oil is fed, at any rate for 54 weeks.

In another experiment 2 rabbits were fed 8 % hydrogenated coconut oil for 21 weeks, with a consequent increase in the serum cholesterol to 787 and 1855 mg/100 ml, respectively. When hydrogenated coconut oil was then replaced by corn oil at the same level and with the same basal diet, the serum cholesterol dropped rapidly to levels of about 250 mg/100 ml (Fig. 10). The animals proceeded on the corn oil diet for altogether 33 weeks, during which the serum cholesterol gradually reached essentially original levels of 79 and 28 mg/100 ml, respectively.

Since hydrogenated coconut oil, which had a pronounced cholesterol-enhancing effect, was rich in lauric acid, an experiment was performed on a small series to test the effect of this fatty acid. Four rabbits were fed on glyceryl trilaurate at the 8 % level for 18 weeks. A striking rise in the serum

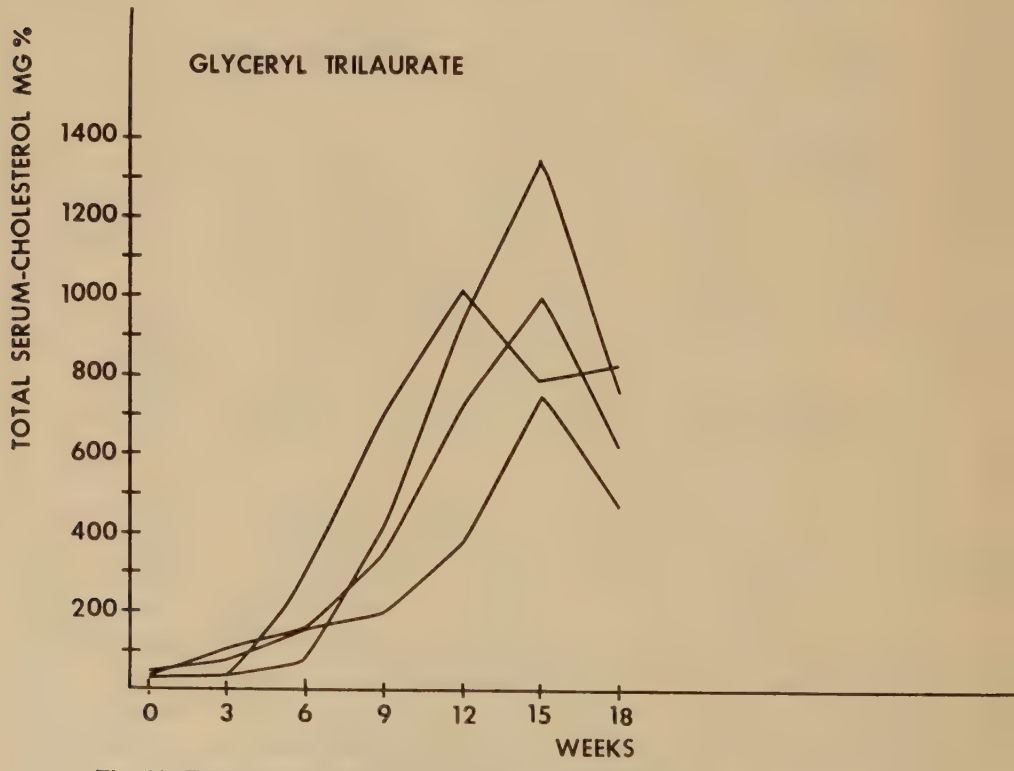

Fig. 11. Total serum cholesterol in 4 rabbits fed 8 % glyceryl trilaurate.

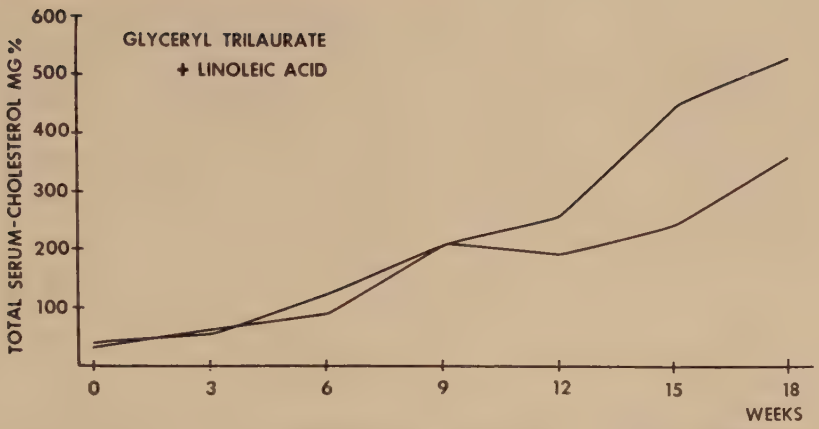

Fig. 12. Total serum cholesterol in 2 rabbits fed 8 % glyceryl trilaurate plus 4 % linoleic acid.

cholesterol was noted in all of the animals, the values reaching a maximum of 742, 999, 1016 and 1343 mg/100 ml, respectively, after 12—15 weeks. The serum cholesterol afterwards decreased again to reach an average value of 665 mg/100 ml at the end of the experiment (Fig. 11).

In order to form an opinion whether linoleic acid might in any way influence the hypercholesterolemic effect of lauric acid, two rabbits were fed on a diet containing 8 % glyceryl trilaurate plus 4 % linoleic acid. It is clear from Fig. 12 that the serum cholesterol rose in both animals, but the increase appeared to be somewhat less pronounced than in the animals that received trilaurate only. The serum cholesterol levels at the end of the experiment after 18 weeks were 535 and 363 mg/100 ml, respectively.

A supplement of 4 % corn oil to the trilaurate diet appeared likewise to be

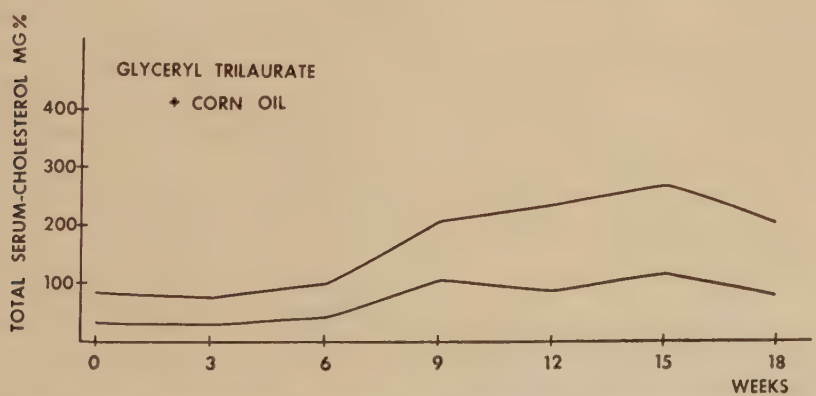

Fig. 13. Total serum cholesterol in 2 rabbits fed 8 % glyceryl trilaurate plus 4 % corn oil.

TABLE 7. *Lipid partition in serum of rabbits before*

Groups	Free cholesterol			Ester-bound cholesterol		
	Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.	Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.
Controls	9.4±0.8	5.6±0.5	—3.9±1.21 $p < 0.025$	16.4±1.3	15.6±1.8	—0.9±2.7 $p > 0.05$
Butter fat	11.2±1.0	93.9±15.1	82.8±14.7 $p < 0.001$	17.6±1.0	249.5±33.4	231.2±32.9 $p < 0.001$
Hydrogenated coconut oil	10.8±1.4	202.8±36.0	192.0±35.4 $p < 0.001$	18.5±2.1	313.9±44.9	295.4±43.4 $p < 0.001$
Corn oil	8.9±0.8	18.9±3.0	9.9±2.6 $p < 0.005$	13.9±0.9	49.1±7.7	35.4±7.3 $p < 0.001$
Rapeseed oil	10.7±0.8	15.2±30.0	4.5±2.9 $p > 0.05$	18.7±1.2	46.9±7.8	28.3±7.0 $p < 0.005$
Glyceryl trilaurate	10.3±1.0	304.0±40.0	293.8±40.6	16.5±0.3	385.3±89.2	368.8±89.2
Glyceryl trilaurate + linoleic acid	11.0±0	151.5±40.5	140.5±40.5	19.5±1.5	228.5±5.5	209.0±7.0
Glyceryl trilaurate + corn oil	25.0±11.0	35.5±15.5	10.5±4.5	23.0±7.0	98.5±42.5	75.5±35.5

Groups	Phospholipids[2]			Total lipids[3]		
	Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.	Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.
Controls	47.0±4.9	37.9±4.8	—9.1±7.8 $p > 0.05$	195.1±23.0	196.4±17.4	1.33±17.2 $p > 0.05$
Butter fat	60.3±4.5	180.2±25.0	119.9±23.9 $p < 0.001$	249.7±32.1	823.5±115.5	573.9±107.9 $p < 0.001$
Hydrogenated coconut oil	42.9±3.0	438.6±61.5	395.7±61.1 $p < 0.001$	183.1±15.1	1952.6±342.5	1769.4±339.0 $p < 0.001$
Corn oil	46.9±3.4	67.8±7.1	20.9±5.8 $p < 0.005$	191.6±19.2	241.6±26.6	50.1±25.0 $p > 0.05$
Rapeseed oil	59.7±4.3	59.1±7.5	—0.6±6.0 $p > 0.05$	231.1±20.7	198.7±30.6	—32.5±31.3 $p > 0.05$
Glyceryl trilaurate	56.5±7.5	468.5±48.5	412.0±55.1	220.8±33.3	1932.0±199.4	1711.3±220.0
Glyceryl trilaurate + linoleic acid	67.5±12.5	295.0±42.0	227.5±29.5	231.0±30.0	1093.5±55.5	862.5±25.5
Glyceryl trilaurate + corn oil	99.5±29.5	111.0±33.0	11.5±3.5	436.5±9.5	419.0±107.0	—17.5±97.5

ınd after feeding different dietary fats (mg/100 ml)

Total cholesterol			Triglycerides [1]		
Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.	Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.
25.9±2.0	21.1±2.2	—4.8±3.8 $p > 0.05$	110.9±19.4	127.0±14.6	16.1±11.0 $p > 0.05$
28.8±1.8	343.4±48.1	314.6±47.5 $p < 0.001$	148.6±26.3	130.3±23.8	—18.3±29.0 $p > 0.05$
29.3±3.1	516.7±77.0	487.4±75.3 $p < 0.001$	97.7±10.7	778.6±212.5	680.9±212.8 $p < 0.01$
22.8±1.5	67.9±10.6	45.1±9.7 $p < 0.001$	112.3±15.3	72.4±10.3	—39.9±16.7 $p < 0.05$
29.4±1.6	62.1±10.7	32.7±9.7 $p < 0.005$	129.4±17.1	45.4±11.2	—84.0±19.7 $p < 0.001$
26.8±1.3	689.3±109.1	662.5±108.6	126.0±26.2	512.0±119.0	386.0±121.4
30.5±1.5	380.0±46.0	349.5±47.5	120.0±20.0	263.5±36.5	143.5±56.5
48.0±18.0	134.0±58.0	86.0±40.0	272.0±45.0	107.0±13.0	—165±32.0

Total cholesterol/Phospholipids		
Before Mean ± S. E.	After Mean ± S. E.	Diff. ± S. E.
0.61±0.08	0.60±0.06	—0.01±0.10 $p > 0.05$
0.49±0.03	1.94±0.13	1.45±0.12 $p < 0.001$
0.69±0.06	1.18±0.07	0.49±0.08 $p < 0.001$
0.50±0.03	0.96±0.08	0.46±0.08 $p < 0.001$
0.51±0.03	1.00±0.09	0.49±0.09 $p < 0.001$
0.50±0.06	1.45±0.10	0.95±0.07
0.47±0.11	1.29±0.03	0.82±0.08
0.47±0.04	1.16±0.18	0.69±0.14

[1] Calculated as glyceryl trioleate.

[2] Lipid phosphorus × 25.

[3] Free cholesterol + cholesteryl esters + triglycerides + phospholipids. Cholesteryl esters were calculated as cholesteryl oleate by multiplying the values for ester-bound cholesterol by 1.68.

accompanied by a decrease in the cholesterol-enhancing effect of lauric acid (Fig. 13). The final values after 18 weeks were 205 and 77 mg/100 ml, respectively. However, owing to the smallness of these groups fed trilaurate, the data were not treated statistically.

Summary

Feeding butter fat or hydrogenated coconut oil at the 8 % level in a cholesterol-free semi-synthetic diet produced a distinct hypercholesterolemia in all rabbits, while feeding corn oil or rapeseed oil at the same level had only a slight effect on the serum cholesterol level. In 3 animals the cholesterol-enhancing effect of hydrogenated coconut oil could be followed for one year. Replacement of hydrogenated coconut oil by corn oil resulted in a rapid disappearance of the hypercholesterolemia and subsequent normalization of the cholesterol levels.

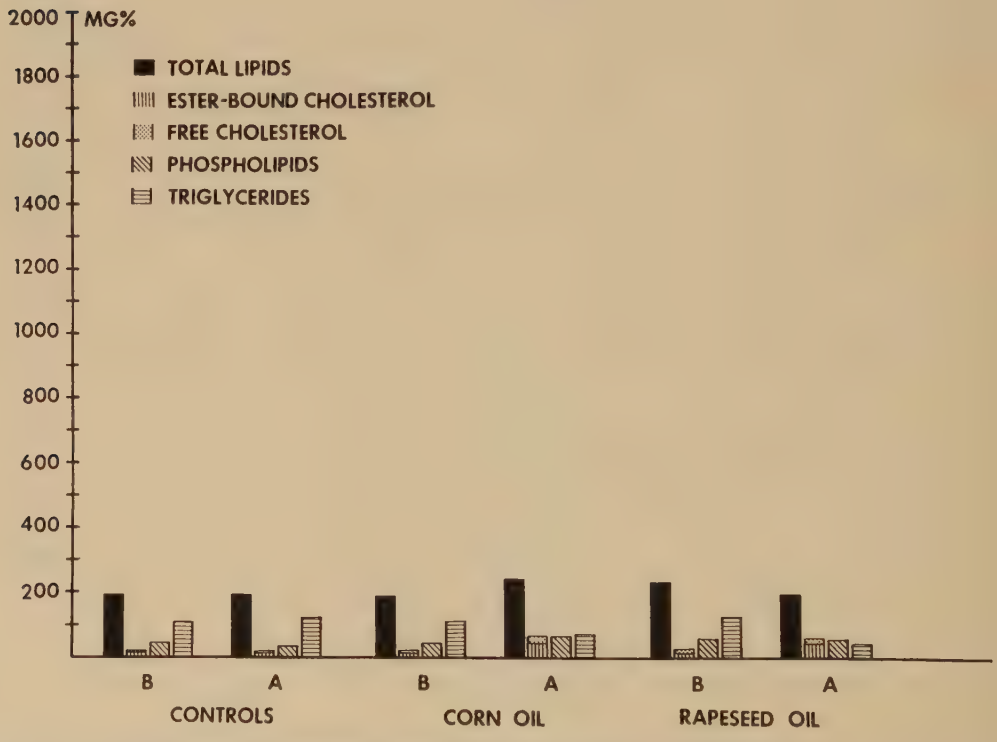

Fig. 14. Lipid partition in serum of rabbits fed 8 % corn oil or rapeseed oil. The controls were fed on green roughage and barley. B=before the feeding period, A=after the feeding period.

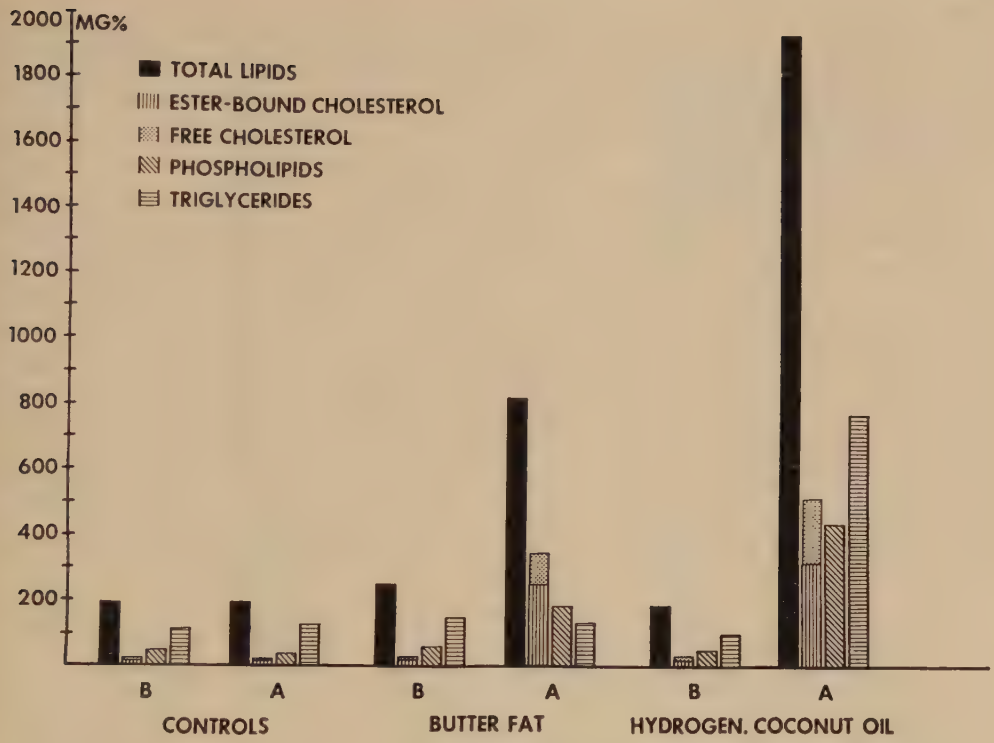

Fig. 15. Lipid partition in serum of rabbits fed 8 % butter fat or hydrogenated coconut oil. The controls were fed on green roughage and barley. B=before the feeding period, A=after the feeding period.

Glyceryl trilaurate fed at the 8 % level resulted in a striking rise of the serum cholesterol level. This effect appeared to be modified to a certain extent if 4 % linoleic acid or corn oil was fed along with trilaurate.

LIPID PARTITION IN SERUM

FREE AND ESTER-BOUND CHOLESTEROL

The average values for the free and ester-bound cholesterol in serum before and at the end of the feeding periods are summarized in Table 7 and are also apparent from Figs. 14, 15 and 16.

In the control group, in which the animals were fed on green roughage and barley, the only significant change observed was a slight decrease in free cholesterol, which was, however, so small that it did not result in any significant change in the total cholesterol level. The ester-bound cholesterol remained unchanged.

In the group fed corn oil a small but statistically significant increase of both the free cholesterol (to 19 mg/100 ml) and the ester-bound cholesterol (to 49 mg/100 ml) was noted while in the rapeseed oil group only a slight increase in the ester-bound cholesterol (to 47 mg/100 ml) was found to be statistically significant.

Compared with the control group, both the corn oil fed and rapeseed oil fed rabbits showed a significantly higher level for free cholesterol ($p<0.001$ and <0.05, respectively) and for ester-bound cholesterol ($p<0.005$ and <0.01, respectively). In both oil-fed groups the cholesterol esters increased more than did the free cholesterol ($p<0.001$).

In the butter fat group and the coconut oil group a distinct increase was noted in both the free and the ester-bound cholesterol in the serum. In the butter fat group the free cholesterol rose from 11 to 94 mg/100 ml and the ester-bound cholesterol from 18 to 250 mg/100 ml. The ratio between free cholesterol and total cholesterol in this group decreased significantly ($p<0.001$) from 0.38 to 0.27 indicating a somewhat larger increase in ester-bound cholesterol compared with that of the free cholesterol.

In the group fed hydrogenated coconut oil the free cholesterol rose from 11 to 203 mg/100 ml and the ester-bound cholesterol from 19 to 314 mg/100 ml. In this group the ratio between free and total cholesterol remained constant ($p>0.05$) indicating an equal relative increase in the two cholesterol fractions.

Comparison between the cholesterol values found in the groups fed butter fat or hydrogenated coconut oil and those found in the control group showed that the differences in the two cholesterol fractions were statistically significant at the 0.1 % level. The butter fat group and the coconut oil group did not differ significantly from one another regarding ester-bound cholesterol. However, the level of free cholesterol was significantly higher in the coconut oil group ($p<0.025$).

Glyceryl trilaurate fed at the 8 % level to 4 rabbits resulted in a striking increase of both the free and the ester-bound cholesterol in the serum in all animals, the average values found for the two fractions at the end of the feeding period being 304 and 385 mg/100 ml, respectively. On feeding the same quantity of trilaurate, but supplemented with 4 % linoleic acid or corn oil the hypercholesterolemic response appeared to be less pronounced in the two rabbits studied on each of these two diets. However, the number of rabbits was too small to permit statistical treatment of the data.

The total serum cholesterol *i. e.* free cholesterol+ester-bound cholesterol in the different groups was of the same order as that found previously on determination of the total cholesterol according to ABELL (see page 32). Thus

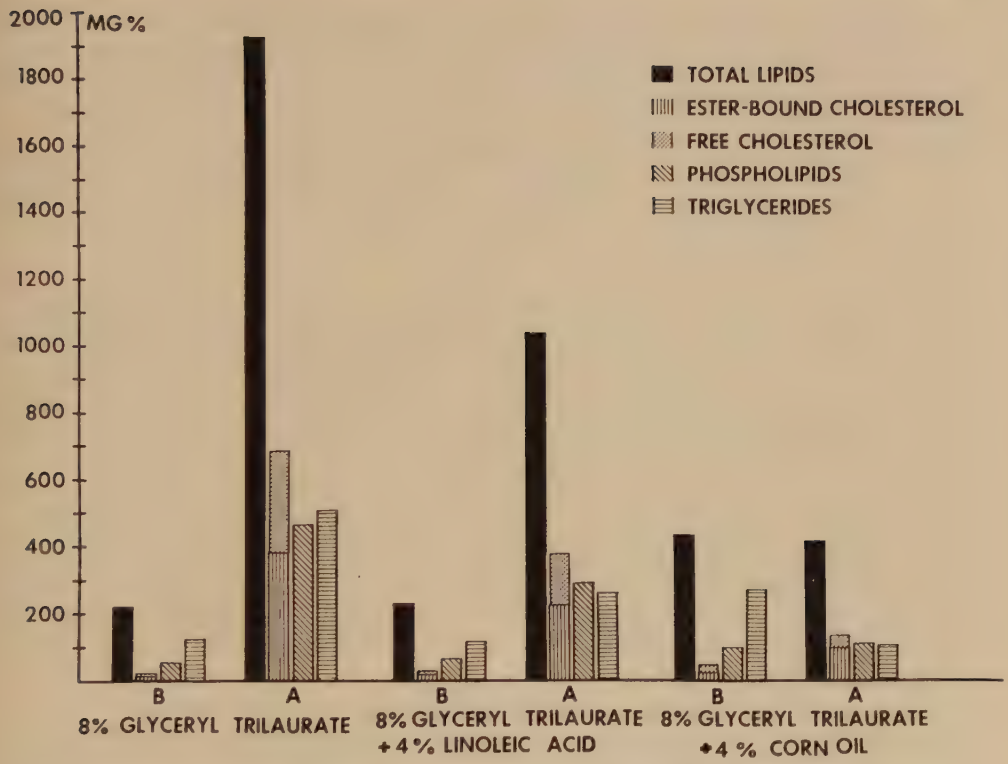

Fig. 16. Lipid partition in serum of rabbits fed 8 % glyceryl trilaurate with and without addition of 4 % linoleic acid or corn oil. B=before the feeding period, A=after the feeding period.

the total cholesterol content in serum at the end of the feeding periods was markedly higher in the group fed hydrogenated coconut oil (517 mg/100 ml) and in the butter fat group (343 mg/100 ml) than in the control group, this difference being statistically significant at the 0.1 % level. The total cholesterol in the corn oil group (68 mg/100 ml) and in the rapeseed oil group (62 mg/100 ml) was found to be only slightly higher than in the control group (21 mg/100 ml), nevertheless, these differences were statistically significant ($p<0.005$ and <0.01, respectively).

Summary

The distinct rise in serum total cholesterol levels observed in rabbits fed 8 % butter fat was found to be due mainly to an increase in the cholesterol ester fraction. In contrast hereto, in the animals fed hydrogenated coconut

oil or glyceryl trilaurate both free and ester-bound cholesterol increased to the same extent. Only a slight increase of the cholesterol fractions was observed in the rabbits fed corn oil or rapeseed oil.

TRIGLYCERIDES

The values found for the triglyceride levels in serum at the beginning and at the end of the feeding periods are summarized in Table 7 and Figs. 14, 15 and 16.

In the different groups studied statistically the average serum triglyceride concentrations at the beginning of the experiment, when the animals were on a green roughage-barley diet, were between 98 and 149 mg/100 ml. In the control group, which continued on this diet throughout the experiment, the triglyceride level remained essentially unchanged, as judged from the average value found after 18 weeks (127 mg/100 ml, $p>0.05$).

In contrast, at the end of the feeding period the group that had received hydrogenated coconut oil showed a pronounced rise in the triglyceride level to an average of 779 mg/100 ml, ($p<0.01$). In most of the animals in this group the serum was increasingly milky from about the middle of the experiment, the milkiness then being most pronounced in those animals with the highest triglyceride level in the serum.

At the end of the feeding period the group that received butter fat in the diet showed an average triglyceride content of 130 mg/100 ml. This did not differ significantly from the original value ($p>0.05$). In none of the animals was lactescence of the serum observed. The difference in serum triglyceride values between the two fat-fed groups at the end of the feeding periods was statistically significant ($p<0.025$) as was the difference between the coconut oil fed rabbits and controls ($p<0.05$). No significant difference was found between the latter group and that fed butter fat ($p>0.05$). In both fat-fed groups a fairly wide variation of individual values was observed.

At the end of the feeding period both the corn oil and rapeseed oil groups showed a fall in the serum triglycerides from 112 to 72 mg/100 ml and from 129 to 45 mg/100 ml, respectively. This decrease was statistically significant for both groups ($p<0.05$ and <0.001, respectively). These two groups, like the control group, never showed milkiness of serum during the experimental period. On comparison with the controls at the end of the feeding periods, both the corn oil fed and the rapeseed oil fed rabbits showed significantly lower triglyceride values ($p<0.05$ and <0.005, respectively).

In all 4 rabbits that received trilaurate, a marked increase was noted in the serum triglycerides at the end of the experiment (from 126 to 512 mg/100 ml). The serum was very milky. The increase in the triglyceride level ap-

peared somewhat less pronounced in those rabbits that received a supplement of 4 % linoleic acid (from 120 to 264 mg/100 ml) while a decrease in the serum triglycerides was observed in both rabbits that were fed on 8 % trilaurate plus 4 % corn oil (from 272 to 107 mg/100 ml). In these 4 rabbits, the serum was not milky. No statistical treatment was done on the data from the groups fed trilaurate owing to the smallness of these groups.

Summary

Various dietary fats fed at the 8 % level resulted in different serum triglyceride levels. Feeding hydrogenated coconut oil was followed by a marked increase of the triglyceride level and milkiness of the serum, while feeding corn oil or rapeseed oil was accompanied by a significant fall in the triglyceride concentration. In contrast hereto, feeding butter fat had no influence on the triglyceride levels.

Feeding glyceryl trilaurate at the 8 % level caused a pronounced rise in the serum triglycerides and lactescence of the serum, while addition of 4 % linoleic acid appeared to modify this effect, and addition of 4 % corn oil apparently resulted in a decrease of serum triglycerides.

PHOSPHOLIPIDS

The values found for the phospholipid concentration in serum at the beginning and end of the feeding periods are summarized in Table 7 and Figs. 14, 15 and 16. In the control group the phospholipid level in serum remained essentially unchanged throughout the experiment. This was also the case in the rapeseed oil group, while in all other groups a significant increase in the phospholipid content was noted. This increase was smallest in the corn oil group (from 47 to 68 mg/100 ml), distinct in the butter fat group (from 60 to 180 mg/100 ml) and most pronounced in the group that received hydrogenated coconut oil (from 43 to 439 mg/100 ml). At the end of the feeding periods the difference between the corn oil group and the control group was found to be statistically significant ($p<0.01$) as was the difference between the latter and the butter fat as well as the coconut oil group ($p<0.001$). Also compared with the butter fat group the phospholipid level in the coconut oil group was found to be significantly higher ($p<0.001$).

A striking increase in the serum phospholipids (from 57 to 469 mg/100 ml), was observed in the rabbits that received 8 % glyceryl trilaurate in the diet, while the increase appeared to be less pronounced in those animals that received trilaurate plus 4 % linoleic acid (from 68 to 295 mg/100 ml) or trilaurate plus 4 % corn oil (from 100 to 111 mg/100 ml).

Summary

The serum phospholipid level rose distinctly in rabbits that received 8 % butter fat as the fat source and still more in those that received hydrogenated coconut oil, while only a slight increase was found in the corn oil fed group. No significant change was observed in the animals fed rapeseed oil. 8 % trilaurate had a markedly enhancing effect on the serum phospholipids, while addition of 4 % linoleic acid or 4 % corn oil to this diet appeared to modify this effect.

CHOLESTEROL/PHOSPHOLIPID RATIO

The values found for the total cholesterol/phospholipid ratio in the serum are given in Table 7. At the beginning of the experiment this ratio was about 0.5—0.7 for all of the groups. At the end of the feeding periods no changes were observed in the control group fed on green roughage and barley, while an increase in this ratio was found for all groups that received additional fat in the diet. This increase was due to a relatively larger increase in the serum cholesterol than in the phospholipids. The largest increase in the cholesterol/phospholipid ratio was found in the group fed butter fat (from 0.49 to 1.94) followed by the coconut oil group (from 0.69 to 1.18) the rapeseed oil group (from 0.51 to 1.00) and the corn oil group (from 0.50 to 0.96). The increases were statistically significant in all these groups ($p<0.001$).

Rabbits that received 8 % glyceryl trilaurate in the diet showed an increase in the cholesterol/phospholipid ratio from 0.50 to 1.45, while the increase appeared to be somewhat less in those animals that received trilaurate plus 4 % linoleic acid (from 0.47 to 1.29) or trilaurate plus 4 % corn oil (from 0.47 to 1.16). However, owing to the smallness of these groups the data were not treated statistically.

Compared with the controls fed on green roughage and barley the difference was statistically significant for corn oil and rapeseed oil groups ($p<0.005$) as well as for those fed butter fat or hydrogenated coconut oil ($p<0.001$). Compared with the group fed butter fat the values for the cholesterol/phospholipid ratio in animals fed corn oil or rapeseed oil were significantly lower ($p<0.001$) while the values in the coconut oil group were at a significantly higher level ($p<0.001$).

Summary

Feeding different fats at the 8 % level resulted in an increase of the total cholesterol/phospholipid ratio in all groups, the values at the end of the feeding periods being highest in those fed butter fat or hydrogenated coconut

oil. Also feeding glyceryl trilaurate at the 8 % level resulted in a marked increase in this ratio.

TOTAL LIPIDS

The values for the total lipid content in serum are given in Table 7 and Figs. 14, 15 and 16. These values represent the sum of the separately determined lipid fractions.

No significant difference was demonstrable in the total lipid concentration before and after the feeding period in the control group fed on green roughage and barley or in the groups fed corn oil and rapeseed oil, respectively. In contrast hereto, a distinct increase in the total lipid content was found in the rabbits fed 8 % butter fat (from 250 to 824 mg/100 ml) and — a still larger increase — in the animals that received hydrogenated coconut oil (from 183 to 1953 mg/100 ml).

Compared with the control group, the hyperlipemia observed in the butter fat and in the coconut oil group was statistically significant ($p<0.001$ and <0.005, respectively). The total lipid level in the serum was also significantly higher in the coconut oil group than in the butter fat group ($p<0.01$). In contrast hereto, no statistically significant difference was found on comparison between the control group on one hand and the corn oil or rapeseed oil group on the other ($p>0.05$).

A pronounced increase in the total lipid level in the serum was also found in the rabbits receiving 8 % glyceryl trilaurate as the only fat source (from 221 to 1932 mg/100 ml), while the increase appeared to be less pronounced in the animals that received 4 % linoleic acid in addition to the trilaurate diet (from 231 to 1094 mg/100 ml). No definite change in the total lipid content in serum appeared to have occurred in the rabbits that were fed trilaurate plus 4 % corn oil (from 437 to 419 mg/100 ml).

Comparison between the changes in the total lipid content and those in the individual lipid fractions in serum revealed that the total lipid level could remain essentially unchanged despite distinct and statistically significant changes in the concentration of the individual lipid fractions. Thus, no significant change was observed in the average total lipid content in serum in the corn oil group, though the cholesterol, the phospholipid and the triglyceride fractions showed statistically significant changes, but of different signs. Similar observations were made in the rapeseed oil group. In the coconut oil group as well as in the butter fat group a pronounced hyperlipemia was observed with an increase in the cholesterol and phospholipid fractions. However, the serum triglycerides increased only in the coconut oil group.

Summary

A pronounced increase in the total lipid content in serum occurred in rabbits whose diet included 8 % butter fat or hydrogenated coconut oil, while no significant change was observed in corn oil or rapeseed oil fed animals. Pronounced hyperlipemia was noted also in those rabbits that received 8 % glyceryl trilaurate as the fat source.

LIPOPROTEINS IN SERUM

The lipoprotein levels in serum noted at the end of the feeding periods are summarized in Table 8. The control group fed the ordinary laboratory diet consisting of green roughage and barley had 36 % α- and 64 % $\beta+0$-lipoproteins. Fairly similar levels were found in the corn oil group (α: 34 %, $\beta+0$: 66 %) and in the rapeseed oil group (α: 31 %, $\beta+0$: 69 %). These values did not differ significantly from those in the control group ($p>0.05$).

In contrast hereto, a distinct relative increase in the $\beta+0$-fraction with corresponding decrease in the α-fraction was found in the group that received 8 % hydrogenated coconut oil in the diet (α: 9 %, $\beta+0$: 91 %). Compared with the control group this difference was statistically significant at the 0.1 % level. The marked increase in the $\beta+0$-fraction was apparently due mainly to a relative increase in the β-lipoproteins. Fig. 17 compares the elpherogram of one rabbit fed hydrogenated coconut oil (above) and of another fed corn oil (below).

In the butter fat group, compared with the control group, there was also a pronounced relative increase in the $\beta+0$-fraction with a corresponding decrease in the α-fraction (α: 13 %, $\beta+0$: 87 %, $p<0.001$).

The difference in serum lipoprotein level between the coconut oil and the butter fat group was not statistically significant ($p>0.05$). The $\beta+0$-fraction in these 2 groups was, however, found to be on a significantly higher level than the corresponding fraction in the rapeseed oil and the corn oil group ($p<0.001$).

A marked relative increase in the $\beta+0$-lipoproteins and a corresponding decrease in the α-lipoproteins occurred also in those rabbits that received 8 % glyceryl trilaurate as the only fat source. No difference appeared to exist between this group and those animals that received a supplement of 4 % linoleic acid or corn oil. These groups were, however, too small to permit statistical analysis of the data.

Based on the data from all experimental groups a statistically positive significant correlation was found between the total cholesterol content of the serum and the magnitude of the $\beta+0$-fraction. The correlation coefficient

TABLE 8

Concentrations (%) of lipoproteins in serum of rabbits fed different dietary fats

Groups	α Mean ± S. E.	β+0 Mean ± S. E.
Controls	35.8±2.8	64.2±2.8
Butter fat	12.8±2.1	87.2±2.1
Hydrogenated coconut oil	8.7±1.3	91.3±1.3
Corn oil	33.8±3.1	66.2±3.1
Rapeseed oil	30.8±2.7	69.2±2.7
Glyceryl trilaurate	2.3±1.0	97.7±1.0
Glyceryl trilaurate + linoleic acid	5.5±4.5	94.5±4.5
Glyceryl trilaurate + corn oil	6.5±0.5	93.5±0.5

between β+0-lipoproteins in serum and the logarithmus of the serum total cholesterol was found to be 0.81, which corresponds to a p-value <0.001.

Summary

Feeding hydrogenated coconut oil or butter fat at the 8 % level resulted in a pronounced relative increase of the β+0-lipoproteins in the serum, while feeding corn oil or rapeseed oil produced no such change. Feeding glyceryl trilaurate resulted in a pronounced rise of the β+0-lipoprotein fraction of the same order as that observed in the coconut oil group.

LIPID PARTITION IN LIVER

FREE AND ESTER-BOUND CHOLESTEROL

The values for free, ester-bound and total cholesterol in liver at the end of the feeding periods are summarized in Table 9. The term "cholesterol" used here means Liebermann-Burchard positive sterols. In rabbit liver the bulk of these sterols consists of cholesterol (GOULD 1951). In the control rabbits that were fed on green roughage and barley the content of total cholesterol (96 μg/mg N) consisted to about 60 % of free cholesterol (57 μg/mg N) and 40 % of ester-bound cholesterol (38 μg/mg N).

A considerably higher total cholesterol concentration was noted in the group that received 8 % butter fat in the diet (409 μg/mg N), this difference,

compared with the control group, being statistically significant at the 0.1 % level. The higher cholesterol level in the butter fat group was due almost entirely to an increase in the ester-cholesterol fraction (346 μg/mg N, $p<0.001$) the concentration of free cholesterol (63 μg/mg N) being at the same level as in the control group ($p>0.05$).

Also in the group that received 8 % hydrogenated coconut oil a distinct increase was noted in the content of total cholesterol (376 μg/mg N, $p<0.001$), which was due mainly to an increase in the ester-cholesterol fraction (272 μg/mg N, $p<0.001$). The concentration of free cholesterol showed only a slight, but significant increase (104 μg/mg N, $p<0.005$) compared with the control group. Comparison between the two fat-fed groups showed that the total cholesterol and ester-cholesterol content in the butter fat and coconut oil group did not differ significantly from one another ($p>0.05$) while the content of free cholesterol was somewhat higher in the coconut oil group than in the butter fat group ($p<0.01$).

In the groups fed corn oil or rapeseed oil about the same concentration of free cholesterol was found as in the control group, namely 54 and 49 μg/mg N, respectively. The ester-cholesterol fraction in these two groups showed a slight increase compared with the controls, the mean values for the corn oil group being 74 μg/mg N and for the rapeseed oil group 104 μg/mg N. This increase was statistically significant ($p<0.025$ and <0.005, respectively). The values for the total cholesterol content were 128 μg/mg N for the corn oil group and 153 μg/mg N for the rapeseed oil group, these values also being significantly higher than in the control group, ($p<0.05$ and <0.01, respectively).

Four rabbits receiving pure glyceryl trilaurate at the 8 % level showed a distinct increase in the ester-bound cholesterol (182 μg/mg N), while the content of free cholesterol (50 μg/mg N) did not appear to differ from that found in the controls. Though the smallness of the series of experimental animals did not permit a statistical analysis of the data, there nevertheless appeared to be a tendency towards lower values for the ester-cholesterol content in those animals that received a supplement either of 4 % linoleic acid (63 μg/mg N) or 4 % corn oil (77 μg/mg N). As the values for free cholesterol in these two groups were rather low (24 and 20 μg/mg N, respectively), the resulting total cholesterol levels apparently did not differ from that found in the control group.

Summary

In adult male rabbits fed on green roughage and barley the total cholesterol content of the liver was found to consist mainly of free cholesterol.

TABLE 9

Lipid partition in liver after feeding different dietary fats determined by chromatographic separation on silicic acid column (μg/mg N)

Groups	Free cholesterol Mean ± S. E.	Ester-bound cholesterol Mean ± S. E.	Total cholesterol Mean ± S. E.	Triglycerides [1] Mean ± S. E.	Phospholipids [2] Mean ± S. E.	Total lipids [3] Mean ± S. E.
Controls	57.2 ± 5.8	38.3 ± 5.4	95.6 ± 8.2	291.1 ± 29.8	820.0 ± 34.4	1232.7 ± 50.7
Butter fat	62.8 ± 10.5	345.9 ± 48.2	408.7 ± 50.3	576.6 ± 94.1	969.6 ± 51.8	2190.2 ± 138.9
Hydrogenated coconut oil	104.0 ± 9.5	272.4 ± 26.3	376.4 ± 30.2	332.6 ± 46.0	702.4 ± 25.5	1596.6 ± 72.8
Corn oil	53.7 ± 3.6	74.0 ± 10.1	127.7 ± 9.2	78.1 ± 17.5	658.1 ± 19.3	914.2 ± 23.1
Rapeseed oil	48.8 ± 3.9	103.7 ± 13.6	152.5 ± 13.5	165.9 ± 28.5	830.3 ± 30.4	1219.1 ± 37.8
Glyceryl trilaurate	50.0 ± 7.1	181.8 ± 28.8	231.8 ± 22.9	274.3 ± 44.6	907.8 ± 18.1	1537.5 ± 82.9
Glyceryl trilaurate + linoleic acid	23.5 ± 0.5	62.5 ± 21.5	86.0 ± 22.0	475.5 ± 77.5	1040.0 ± 30.0	1626.0 ± 144.0
Glyceryl trilaurate + corn oil	19.5 ± 3.5	77.0 ± 41.0	96.5 ± 37.5	245.5 ± 96.5	986.0 ± 69.0	1380.5 ± 37.5

[1] Calculated as glyceryl trioleate.

[2] Lipid phosphorus × 25.

[3] Free cholesterol + cholesteryl esters + triglycerides + phospholipids. Cholesteryl esters were calculated as cholesteryl oleate by multiplying the values for ester-bound cholesterol by 1.68.

Feeding 8 % butter fat or hydrogenated coconut oil in a semi-synthetic basal diet was followed by a pronounced increase of ester-bound cholesterol, while free cholesterol showed no change or only a slight change. Rapeseed or corn oil fed rabbits showed only slight changes, the concentration of the ester-bound cholesterol being somewhat higher than in the control animals. 8 % glyceryl trilaurate produced a distinct increase in the ester-bound cholesterol, while a supplement of linoleic acid or corn oil to the diet appeared to modify this increase.

TRIGLYCERIDES

The values found for the triglyceride content in the liver at the end of the feeding periods are summarized in Table 9.

In the control group, fed on green roughage and barley the triglyceride level was 291 μg/mg N. Feeding 8 % butter fat in the semi-synthetic basal diet was followed by a moderate accumulation of triglycerides in the liver, the values being on a significantly higher level (577 μg/mg N) than in the control group ($p<0.025$). In contrast hereto, compared with the control group, a rather pronounced decrease in the triglyceride content was noted in the corn oil group (78 μg/mg N, $p<0.001$) and in the rapeseed oil group (166 μg/mg N, $p<0.01$). Hydrogenated coconut oil had no certain effect on the triglyceride fraction (333 μg/mg N, $p>0.05$). The accumulation of triglycerides observed in the butter fat group was statistically significant also when compared with the coconut oil group ($p<0.025$). It is also clear from the statistical analyses that the triglyceride-depressing effect of corn oil was somewhat greater than that of rapeseed oil ($p<0.025$).

Feeding glyceryl trilaurate at the 8 % level did not appear to influence the triglyceride content (274 μg/mg N), and neither did the addition to this diet of 4 % corn oil. The two animals receiving 8 % trilaurate plus 4 % linoleic acid showed, on the other hand, somewhat higher values. However, as the smallness of these latter series did not permit a statistical analysis of the data the significance of this finding is not clear.

Summary

Feeding 8 % butter fat in the semi-synthetic basal diet resulted in a moderate accumulation of triglycerides in the liver, while feeding corn oil or rapeseed oil depressed this fraction. Hydrogenated coconut oil had no statistically significant effect. Feeding glyceryl trilaurate did not appear to change the triglyceride concentration.

PHOSPHOLIPIDS

The values for the phospholipid content of the liver at the end of the feeding periods are given in Table 9.

The phospholipid fraction represented the quantitatively largest part of the liver lipids in all rabbits studied. In the control group, that was fed on a green roughage-barley diet, this fraction was found to be on the average 820 μg/mg N.

Feeding different fats at the 8 % level produced at most a moderate, if any, difference in the phospholipid fraction. Compared with the control group, the phospholipid fraction was unchanged in the rapeseed oil group (830 μg/mg N, $p>0.05$), while a moderate increase was noted in the butter fat group (970 μ/mg N, $p<0.05$) and a moderate decrease was found in the coconut oil group (702 μg/mg N, $p<0.025$). A somewhat more pronounced fall was noticed in the corn oil group (658 μg/mg N, $p<0.001$).

Also in the rabbits that received glyceryl trilaurate, trilaurate plus linoleic acid or trilaurate plus corn oil in the diet the phospholipid concentration was of the same order as in the control group, though the values appeared to be at a somewhat higher level. The smallness of these series did not, however, permit a statistical analysis of the data.

Summary

The phospholipids represented the quantitatively largest part of the liver lipids. Feeding different fats at the 8 % level induced at most moderate changes, the values being somewhat higher in the animals fed butter fat and lower in those fed hydrogenated coconut oil or corn oil.

TOTAL LIPIDS

The total lipid content of the liver at the end of the feeding periods is apparent from Table 9. The values were calculated as the sum of the individually determined lipid fractions.

The total lipid content found in the livers of the control rabbits averaged 1233 μg/mg N. Compared with this level, feeding 8 % butter fat or hydrogenated coconut oil produced an increase of the lipid content of the liver. The increase was most pronounced in the butter fat group (2190 μg/mg N, $p<0.001$), while the coconut oil group showed only a moderate, but nevertheless significant increase (1597 μg/mg N, $p<0.005$). Feeding rapeseed oil did not produce any significant change in the total lipid content (1219 μg/mg N, $p>0.05$), while corn oil produced a moderate decrease in the total lipid level (914 μg/mg N, $p<0.001$).

A statistically significant difference was found between the butter fat and

the coconut oil group ($p<0.001$), between the coconut and the rapeseed respectively the corn oil group ($p<0.001$) and between the rapeseed oil and the corn oil group ($p<0.001$).

Comparison of these figures with the differences observed in the individual lipid fractions between the various groups shows that determination of the total lipid content alone does not permit valid conclusions concerning changes in the lipid fractions. The changes observed occured mainly in the cholesterol ester and triglyceride fraction, while free cholesterol and phospholipid content remained somewhat more constant. That an increase in the total lipid content need not imply any increase in the triglyceride content is obvious from the results obtained in the coconut oil group, where the triglyceride content was unchanged compared with the control group, the increase in total lipids being mainly due to a pronounced increase in the cholesterol ester fraction.

A moderate increase in the total lipid content was observed also in the rabbits that received 8 % glyceryl trilaurate with or without addition of 4 % linoleic acid or corn oil. However, owing to the smallness of these groups, no statistical analysis of the data was carried out.

Summary

Feeding 8 % butter fat or hydrogenated coconut oil produced an increase in the total lipid content of the liver, which was most pronounced in the butter fat group. Rapeseed oil produced no change, while corn oil resulted in a lowering of the total lipid content.

FAT EXCRETION IN FECES

The values for excretion of non-saponifiable fraction and total fatty acids in the feces are summarized in Table 10. The values were corrected for excretion of "metabolic lipid", *i. e.* the fatty materials present in bacterial residues, digestive juices and secretions, and in the epithelial cells of the stomach and intestinal tract, which will be present in the feces along with any undigested fat (Deuel 1955). This was done by substracting the quantity of the non-saponifiable fraction and total fatty acids excreted in a comparable group of 7 rabbits receiving a fat-free basal diet similar to that used in the experimental groups except that in this diet the fat had been substituted by carbohydrates and starch. It is not known whether the amount of metabolic lipid is exactly the same in rabbits irrespective whether fat-free or fat-containing diets are fed, as the higher carbohydate content in the fat-free diet, for example, might possibly affect the intestinal bacterial

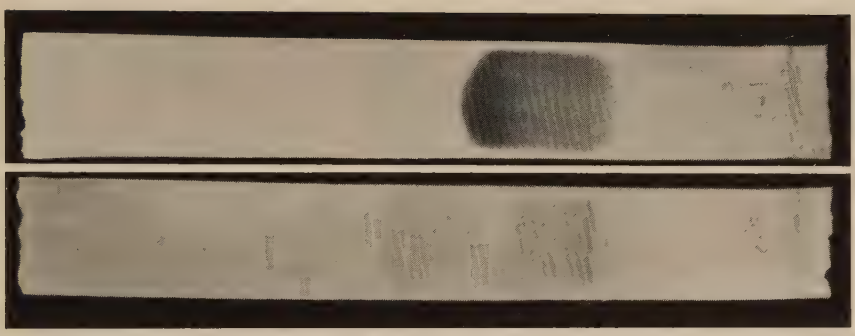

Fig. 17. Distribution of serum lipoproteins after electrophoretic separation on filter paper.
Above: rabbit fed 8 % hydrogenated coconut oil for 15 weeks,
Below: rabbit fed 8 % corn oil for 15 weeks.

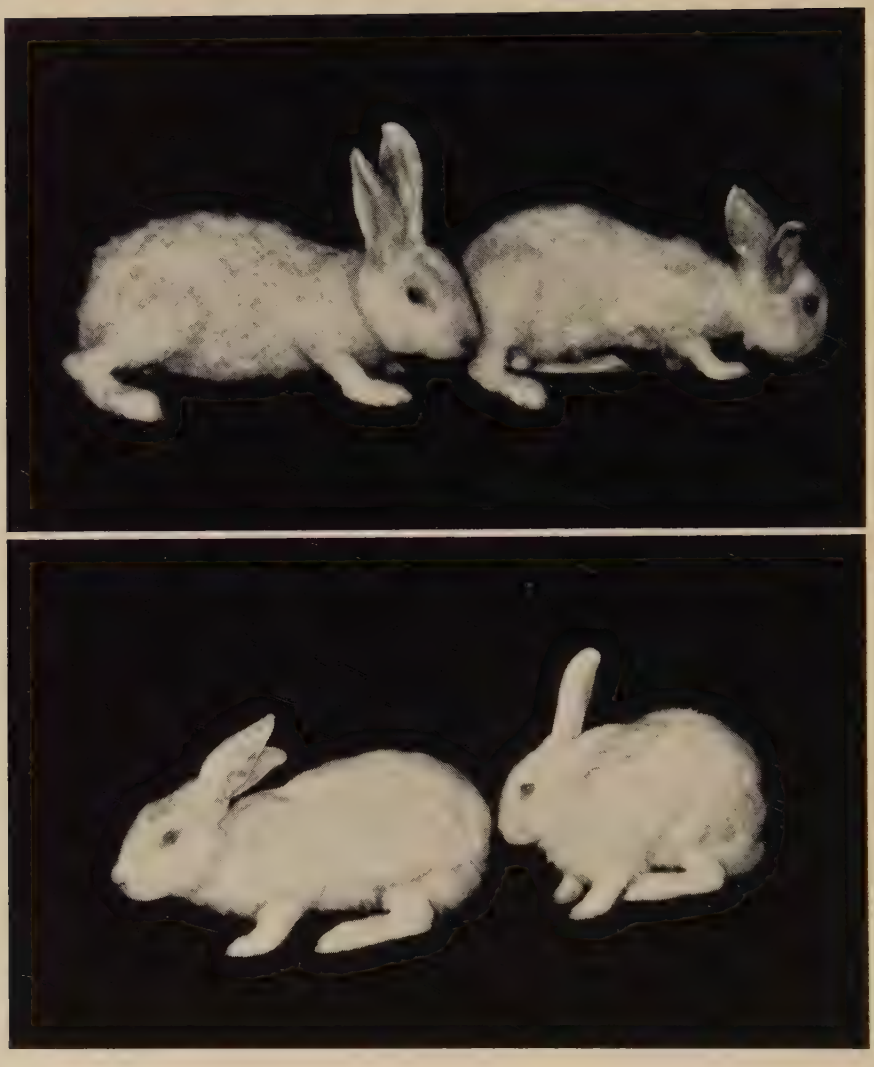

Fig. 18. Appearance of rabbits fed 8 % hydrogenated coconut oil (above) or 8 % corn oil (below) for 15 weeks.

Fig. 19. Appearance of aorta from a rabbit fed 8 % hydrogenated coconut oil (left) and from a rabbit fed 8 % corn oil (right) for 15 weeks. After staining with Sudan IV.

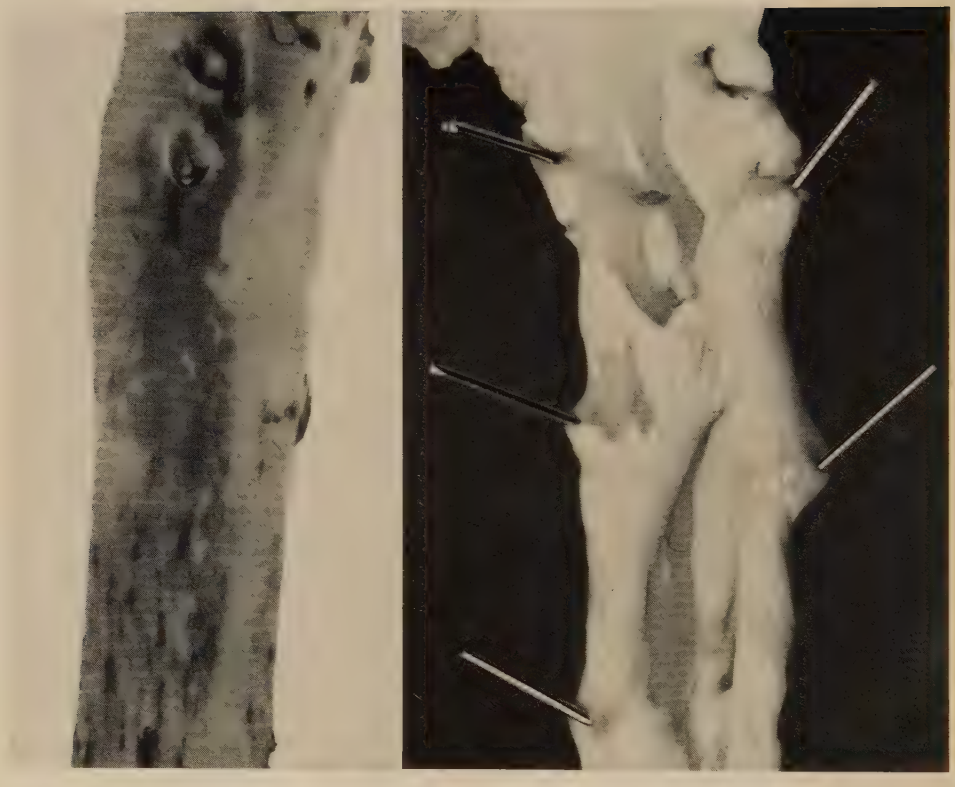

Fig. 20. Appearance of aorta from a rabbit fed 8 % butter fat for 18 weeks (left, after staining with Sudan IV) and from a rabbit fed 8 % hydrogenated coconut oil for 54 weeks (right, unstained).

TABLE 10

Excretion of non-saponifiable fraction and total fatty acids in feces of rabbits fed different dietary fats. The values are expressed as mg per kg body-weight per day [1]

Groups	Non-saponifiable fraction Mean ± S. E.	Total fatty acids Mean ± S. E.	Absorption percentage
Controls	101.30±10.12	70.00±10.92	—
Butter fat	3.69±0.85	25.73±9.84	99.1
Hydrogenated coconut oil	12.81±0.74	41.63±5.50	98.0
Corn oil	29.21±1.44	17.39±1.95	99.2
Rapeseed oil	29.02±2.54	110.26±17.60	94.3
Glyceryl trilaurate	3.48±1.05	11.38±2.68	99.7
Glyceryl trilaurate + linoleic acid	4.90±2.30	10.12±2.25	99.9
Glyceryl trilaurate + corn oil	10.70±1.79	23.35±9.95	99.2

[1] The values for the fat-fed groups are corrected for excretion of metabolic lipid determined in 7 rabbits on fat-free semi-synthetic basal diet. In this group the following mean values were found: non-saponifiable fraction: 6.30 mg/kg/day; total fatty acids: 7.70 mg/kg/day.

flora resulting in altered lipid excretion (PORTMAN et al. 1956 c). In spite of this, such correction was believed to adjust the values for the experimental groups in the right direction.

As is apparent from the table, compared with the control group fed green roughage and barley, the amounts of non-saponifiable fraction and total fatty acids excreted were rather small in all groups fed the semi-synthetic basal diet. The values found for excretion of total fatty acids were used for the calculation of the fat absorption. The absorption percentages (see page 25) found for the different groups revealed that the absorption of the fats supplied was satisfactory and of roughly the same order. The lowest average value, 94.3 %, was found in the rapeseed oil group.

From the values for the excretion of non-saponifiable fraction it is clear that this was somewhat larger in the corn oil and rapeseed oil group compared with the coconut oil group ($p<0.001$) and compared with the butter fat group ($p<0.001$). No difference appeared to exist between the excretion of non-saponifiable fraction in the group fed 8 % glyceryl trilaurate and that fed trilaurate plus linoleic acid, the amounts excreted being

very small. Somewhat larger values were found in the group that received trilaurate plus corn oil. These groups of experimental animals, however, were not large enough to permit statistical analysis of the data.

Summary

From the determination of the total fatty acid content in the feces at the end of the feeding periods the absorption percentage of the different dietary fats was calculated and found to vary between 94 and 100 %.

Rabbits fed corn oil or rapeseed oil excreted a somewhat larger amount of non-saponifiable fraction per kg body-weight than rabbits fed butter fat or hydrogenated coconut oil.

DERMAL SYMPTOMS

From about the middle of the feeding periods animals fed butter fat or hydrogenated coconut oil began to loose hair. This loss of hair usually began on the inner side of the hind leg and on the abdomen. During the further course of the experiment the loss became more and more conspicuous and in some cases spread out over the back of the animal and the tail, and to the fore and hind legs, but not to the paws. Neither was the head involved, which in those animals in which the loss of hair was greatest, then appeared to be abnormally large and heavy for the neck, which, owing to loss of hair, appeared to be very thin. Fig. 18 (above) gives two examples of the most advanced changes observed in animals fed hydrogenated coconut oil.

Pronounced loss of hair over the major part of the body was observed at the end of the feeding periods in 10 of the 18 rabbits in the coconut oil group and in 4 of the 13 rabbits in the butter fat group. A further 2 rabbits in the coconut oil group and 2 rabbits in the butter fat group showed moderate loss of hair, particularly on the abdomen. In contrast to these conspicuous changes, only slight loss of hair, which passed off after 2—3 weeks, was observed towards the end of the experiments in most of the rabbits in the corn oil and rapeseed oil group and in the control group, which was on the green roughage-barley diet. These changes appeared to represent a normal and physiologic regeneration of the fur, since a similar loss of hair was observed at the same time in other rabbits kept in the same stable. The loss of hair was not very pronounced and apart from this period, the animals had a shiny fur of normal appearance, even at the end of the experiment. Fig. 18 (below) shows the appearance of 2 rabbits after having been on a corn oil diet for 15 weeks.

No definite correlation was found between the severity of the loss of hair

and the serum cholesterol level. However, two of the three rabbits in the coconut oil group that had the most pronounced hypercholesterolemia showed excessive loss of hair over the entire body at the end of the experiment.

Moderate to severe loss of hair was observed also in the 4 rabbits that received glyceryl trilaurate in the diet, while no such changes occurred in the animals that also received a supplement of linoleic acid or corn oil.

Two of three rabbits that had been fed hydrogenated coconut oil for 54 weeks showed considerable loss of hair on the abdomen and bilaterally on the back after 4—5 months. However, about 20 weeks later the hair began to grow again and, after a further 8 weeks (about 50 weeks from the beginning of the experiment), the hair was of almost normal appearance in both animals. During the entire period they had received the same diet containing 8 % hydrogenated coconut oil.

No other dermal symptoms such as scaly skin, caudal degeneration or secondary infections were observed in the experimental groups. Neither could any xanthomatous nodules be demonstrated.

Summary

Animals fed 8 % hydrogenated coconut oil, butter fat or glyceryl trilaurate in the semi-synthetic diet showed an increasing loss of hair for the 15—18 weeks the experiment lasted. On the other hand, feeding corn oil or rapeseed oil did not produce such changes. Spontaneous regression of the fur changes was observed in 2 full-grown rabbits fed hydrogenated coconut oil for one year.

AORTIC CHANGES

The control rabbits, which were fed on green roughage and barley, showed no gross sudanophilic changes in the aorta at the end of the experimental period, *i. e.* after 18 weeks.

A picture similar to that seen in the control group was observed in the groups that received corn oil or rapeseed oil in the diet. In these animals, too, the aortic wall was macroscopically normal with a smooth and shiny surface and showed no stainable changes (Fig. 19, right).

In contrast to these negative findings in rabbits fed green fodder or fluid vegetable oils, distinct macroscopic changes were observed in the aorta of most rabbits that received 8 % butter fat or hydrogenated coconut oil added to the cholesterol--free basal diet. Already in the unstained specimen distinct changes were seen resembling the atherosclerotic lesions observed earlier in

cholesterol-fed rabbits. When stained with Sudan IV, these changes consisted of intensely dark red, well defined patches or streaks showing up distinctly against the faintly stained background. In those animals which showed only slight changes these were observed, as a rule, in the ascending and thoracic aorta only, predominantly localized around the origin of the intercostal arteries. In the more advanced cases the aortic changes were more widespread and consisted of large, often confluent and elevated plaques localized over the entire length of the aorta and even on the heart valves. The most pronounced changes were observed in 3 rabbits that had been fed hydrogenated coconut oil. A typical example is shown in Fig. 19 (left). The changes seen in the butter fat group were as a rule somewhat more moderate and are illustrated by the specimen shown in Fig. 20 (left).

Gross lipid deposits in the aorta of the type described above were observed in altogether 12 of 13 rabbits in the butter fat group and in 16 of 18 rabbits in the coconut oil group (see Table 11). The 3 rabbits in these groups that showed no gross changes had only a moderate hypercholesterolemia. To these animals belonged the rabbit in the coconut oil group that appeared in a certain way "refractory" in that its serum cholesterol did not rise appreciably until much later than that in the other animals belonging to this group.

That the aortic changes observed after the rabbits had been on the experimental diet for 15—18 weeks might progress on prolongation of the fat feeding is suggested by the changes observed in one rabbit that had received 8 % hydrogenated coconut oil in the diet for 54 weeks. The major part of the aorta from the heart valves down to the bifurcation was covered with thick mounded confluent yellow-white plaques (Fig. 20, right).

Gross aortic lipid deposits were seen also in those rabbits that received 8 % glyceryl trilaurate with or without addition of 4 % linoleic acid while no changes were observed in the 2 rabbits that had received trilaurate plus 4 % corn oil (see Table 11).

The present investigation did not include histologic examination of the material. That part will be published elsewhere.

The statistical analysis, which is based on data from the entire material, showed a statistically significant positive correlation between the concentration of free, ester-bound and total cholesterol in serum and the severity of the aortic changes. The corresponding correlation coefficients (r) in the above order were found to be 0.77, 0.82 and 0.82, respectively. These values correspond to p-values <0.001. A statistically significant positive correlation was also found between the order of the cholesterol phospholipid ratio in serum and the severity of the aortic changes, the correlation coefficient being 0.54 ($p<0.001$). While all rabbits in which aortic lesions were noted had in-

TABLE 11

Average terminal grading[1] *of gross aortic lesions in rabbits fed different dietary fats. After staining with Sudan IV*

Rabbit No.	Controls	Butter fat	Hydrogenated coconut oil	Corn oil	Rapeseed oil	Glyceryl trilaurate	Glyceryl trilaurate + linoleic acid	Glyceryl trilaurate + corn oil
1	0	+	++	0	0	+	++	0
2	0	++	+	0	0	+	+	0
3	0	+	+	0	0	+		
4	0	+	+	0	0	++		
5	0	++	+	0	0			
6	0	+	+++	0	0			
7	0	++	0	0	0			
8	0	++	+	0	0			
9	0	+	+++	0	0			
10		++	+	0	0			
11		+	+++	0	0			
12		0	+	0	0			
13		+	0	0	0			
14			+	0	0			
15			++	0	0			
16			+	0				
17			+					
18			+					

[1] Se page 25.

creased serum cholesterol and phospholipid levels the triglyceride concentration was increased only in those animals that had been fed on hydrogenated coconut oil or glyceryl trilaurate. In the butter fat group normal triglyceride levels were the rule. However, most of these animals showed sudanophilic deposits in the aorta. It was therefore considered incorrect to calculate the correlation between serum triglyceride values and aortic changes on the base of the values from the entire material. In the group fed on hydrogenated coconut oil the animals which showed the most advanced aortic lesions had the highest triglyceride levels.

Summary

Feeding butter fat or hydrogenated coconut oil at the 8 % level added to a cholesterol-free semi-synthetic basal diet resulted in gross aortic changes similar to cholesterol-induced atherosclerosis in about 90 % of the rabbits studied, while animals fed corn oil or rapeseed oil showed completely normal aortae. Feeding glyceryl trilaurate with or without supplementary linoleic

acid also resulted in aortic lipid deposits, while rabbits fed trilaurate plus corn oil showed no such changes.

A significant positive correlation was found to exist between the level of free, ester-bound and total cholesterol as well as the total cholesterol/phospholipid ratio in serum and the incidence and severity of the aortic changes.

Chapter VI

DISCUSSION

Since the fundamental experiments by ANITSCHKOW (ANITSCHKOW 1912, ANITSCHKOW & CHALATOW 1913) cholesterol feeding has been used as the method of choice in the experimental production of hypercholesterolemia and atherosclerosis. However, in the study of the atherogenic effect of different dietary fats, it cannot be regarded as really satisfactory to administer cholesterol, since cholesterol by itself, *i. e.* fed without fat, has been found to produce hypercholesterolemia and atherosclerosis (POPJAK 1946). During the last few decades it has generally been regarded as established that atherosclerosis cannot be induced in rabbits without administration of cholesterol (DUFF 1935, COOK 1958). This concept, originally based on the negative results obtained by ANITSCHKOW (1913), VERSÉ (1917) and DUFF (1936) on feeding fluid vegetable oils in cholesterol-free diets has apparently received support by recent investigations.

Thus, PUTIGNANO (1952) found no aortic changes in rabbits fed olive oil, boiled or not, added to the green-fodder diet. HIRSCH & NAILOR (1955) found hyperlipemia, mainly as an increase in neutral fat, but no lipid deposits in the aorta of rabbits fed for 180 days on a regular diet with an addition of 15—50 cc cream. POLLAK (1956) fed rabbits on Purina chow plus 10 ml cottonseed oil for 14 days and observed only transient and moderate hypercholesterolemia. No lipid deposits could be demonstrated in the aorta. CHAKRAVARTI et al. (1956) found no lipid deposit in any artery of groups of 6 rabbits fed 5 ml of commercial cottonseed, coconut or groundnut oil a day for 28 weeks in addition to a diet consisting of gram, bran and green vegetables. No rise in the serum cholesterol was observed. CARROLL (1957) reported that feeding of 25 % rapeseed oil in a Purina chow diet did not result in formation of plaques in the aorta or coronary arteries in male rabbits. Neither did this diet affect the serum or tissue cholesterol level. VAN HANDEL & ZILVERSMIT (1957) fed rabbits on Purina chow plus 35 % liquid cottonseed oil, selectively hydrogenated cottonseed oil or corn oil. After 4 months on the high-fat regimen serum cholesterol or phospholipid concentrations were no higher than at the start. Isocaloric substitution of hydrogenated vegetable

oil for liquid cottonseed or corn oil had not any cholesterolemic effect. No gross atheromatous lesions were noted in the aorta.

In these experiments the basal diet invariably consisted either of green fodder or commercial chow. The latter may, however, contain up to 4 % ether-choloroform extractable material (KRITCHEVSKY et al. 1954). Such fat content cannot be regarded as insignificant in nutritional experiments of this type, especially as the fatty acid composition of the fat was unknown, and might possibly have interfered with the fat to be tested. More than 50 % of the fatty acid content of the green roughage, barley and mangels used in the present investigation was found to consist of polyunsaturated fatty acids.

In studies on the vehicle effect of dietary fat in rabbits KRITCHEVSKY et al. (1954) found partially hydrogenated vegetable oil (commercial shortening) or corn oil fed at the 9 % level in a cholesterol-free Purina chow diet to have no effect on serum lipids or aorta atheroma. However, in animals fed 3 % cholesterol some difference was observed in that shortening-fed animals had a mean serum cholesterol level of 3990 mg/100 ml, while rabbits fed corn oil had 3150 mg/100 ml. While it is not stated whether this difference was statistically significant, a significant difference was found in the severity of aortic lesions, the shortening-fed rabbits showing an index of 3.7 and the corn oil fed animals an index of 2.7. These results suggested the possibility that in cholesterol-fed rabbits different dietary fats might modify the hypercholesterolemic response and the incidence of atherosclerosis in a different manner.

In 1956 STEINER & DAYTON found that, under certain conditions, the dietary fat does by itself influence the lipid metabolism and appearance of the aorta in rabbits. These authors succeeded in producing moderate hypercholesterolemia in full-grown male rabbits by feeding them on a cholesterol-free Purina chow diet containing 50—75 % ground peanuts. After 10 to 12 months the authors observed one gross atherosclerotic plaque in 2 of the 33 animals studied. However, microscopic lipid deposits in the intima and underlying media of the ascending and thoracic aorta were seen in 10 rabbits. The fat represented as much as 40 % in this diet.

In the present investigation adult rabbits were chosen as the experimental animals, since of all laboratory animals that have been tried (COX et al. 1958, KATZ et al. 1959) hypercholesterolemia and atherosclerosis have been produced regularly and easily in this species (ANITSCHKOW 1913, DUFF 1935, HUEPER 1944, 1945) and more readily in fullgrown than in young animals (POLLAK 1947). Even relatively small amounts of cholesterol are sufficient to produce such changes if fed long enough (ANITSCHKOW 1933). Also the chick has proved suitable for such studies (DAUBER & KATZ 1942, KATZ & STAMLER

1953). Other species, such as rats, hamsters, cats and dogs have proved fairly refractory to exogenous cholesterol (ANITSCHKOW 1913, DUFF 1935, HORLICK & HAVEL 1948, GOLDMAN & POLLAK 1949, KATZ 1952) and, in order to produce aortic lesions, cholesterol feeding has had to be supplemented with rather drastic methods such as induction of hypothyroidism (STEINER & KENDALL 1946, MARX et al. 1950, PAGE & BROWN 1952, MALINOW et al. 1953, FILLIOS et al. 1956), deficiency of lipotropic factors, such as choline (HARTROFT et al. 1952) or preceding physical, chemical or toxic irritation of the vessel wall (TAYLOR 1955, WATERS 1955, GÜNTHER 1958). In monkeys hypercholesterolemia and atherosclerosis have been produced by diets deficient in certain essential food-stuffs, such as pyridoxine or sulphur-containing amino acids (RINEHART & GREENBERG 1948, 1949, MANN et al. 1953) but also by feeding large amounts of fat plus cholesterol for 4 years (MANN & ANDRUS 1956). The susceptibility of the guinea-pig to exogenous cholesterol has been found to vary (COOK & MCCULLAGH 1939, ALTSCHUL 1950 b).

Experiments carried out at this laboratory during 1956 showed no significant difference in the effect of corn oil or hydrogenated coconut oil on the severity of hypercholesterolemia or atherosclerosis in rabbits fed cholesterol in an alfalfa-wheat-soybean meal diet supplemented with kale (MALMROS & WIGAND, unpublished data). Therefore, in the present investigation it was decided to use a cholesterol-free, semi-synthetic basal diet. It was assumed that such a diet would also make it possible to keep the dietary fat at a moderate level.

With this diet and the fat added representing 8 % by weight, which corresponds to 26 % of the calories, significant effects of different fats could be demonstrated. Thus, feeding butter fat or hydrogenated coconut oil to adult male rabbits for about 4 months resulted in a distinct hypercholesterolemia with subsequent gross changes in the aorta resembling those seen in cholesterol-induced atherosclerosis. Feeding rapeseed oil or corn oil, on the other hand, produced only slight changes in the serum cholesterol and no aortic lipid deposits. This absence of lipid deposition in the aorta is in agreement with the negative results obtained in the earlier experiments referred to on page 63, in which fluid vegetable oils had been fed in cholesterol-free diets.

In 1958, while the study was in progress, independently of the present author, LAMBERT et al. published investigations using an essentially similar experimental design. They found that feeding 20 % hydrogenated coconut oil in a cholesterol-free semi-synthetic diet to adult male rabbits for 100 days produced a hypercholesterolemia of about 730 mg/100 ml and subsequent atheromatous lesions in the aorta in all animals, while 20 % safflower oil resulted in only a slight increase in serum cholesterol, and in this group only

4 of 10 rabbits showed any aortic involvement, as manifested by slight sudanophilia.

The present results as well as those reported by LAMBERT et al. indicate that different dietary fats *per se* do exert different effects on the lipid metabolism in the rabbit and that they may have different atherogenic properties. The suggestion that this effect may in some way or other be masked if the basal diet contains even a small percentage of some other fat receives support by very recent results reported by STEINER et al. (1959), who found only a slight rise of the serum cholesterol to 110 mg/100 ml and no aortic changes in rabbits fed 12 % coconut oil without supplementary cholesterol. The basal diet in that experiment consisted of Purina chow, which contained 2 % vegetable fat.

During the last few years the influence of different dietary fats on lipid metabolism and atherogenesis has been studied in other species, too. The results have been contradictory and will only be briefly mentioned here. Thus, in the rat several authors have found that relatively saturated fats such as lard, butter fat, coconut oil, hydrogenated peanut oil, hydrogenated cottonseed oil and Crisco produced an increase in the serum cholesterol compared with unsaturated fats, such as corn oil, soybean oil, safflower oil, cod-liver oil, cottonseed oil and oleic acid. (SWELL & FLICK 1953, WISSLER et al. 1953, BROMER & DAY 1953, SHAPIRO & FREEDMAN 1955, AFTERGOOD et al. 1956, AVIGAN & STEINBERG 1957, GRUNBAUM et al. 1957, BYERS & FRIEDMAN 1958, HAUGE & NICOLAYSEN 1959, SESKIND et al. 1959, DE GROOT & REED 1959.) On the other hand, SWELL et al. (1955) found slightly higher serum cholesterol values with increasing iodine values of soybean oil hydrogenated to varying degrees. Somewhat similar results were obtained by KLEIN (1958). DILLER et al. (1957) and BUTTNER & MUHLER (1958) found that plasma cholesterol was not affected by the degree of unsaturation of the dietary lipid, which is in agreement with the finding of BEST et al. (1958), who could not show any significant difference in the total serum cholesterol of rats fed 10 edible oils and fats without cholesterol. In studies of 65 different oils and combinations of oils HEGSTED et al. (1957 a, 1957 b) found an inverse relationship between the serum total cholesterol level and the product obtained by multiplying the saturated fatty acid content of the diet by the linoleic acid content. While these authors did not study the aorta, WISSLER et al. (1953) found lipomatous coronary artery changes in rats fed a high-fat diet containing lard, but not in animals fed corn oil. MALINOW et al. (1954) reported atheromatous changes after feeding rats on a bread-milk-meat diet plus 4 ml sunflower oil. In both of these experiments the basal diet was cholesterol-free.

In the cholesterol-fed cebus monkey, PORTMAN et al. (1956 a, 1956 b) found higher serum cholesterol levels on feeding hydrogenated cottonseed oil than on feeding corn oil at various levels and a lowering of serum cholesterol levels on replacement of hydrogenated by unhydrogenated cottonseed oil. COX et al. (1958) found an increase in the serum cholesterol in Rhesus monkeys fed 42 % of the calories as butter or margarine in a cholesterol-free diet.

In mice fed a cholesterol-free basal diet consisting of oats and skim milk SCHETTLER (1949) found increased serum and organ cholesterol levels on feeding animal fat compared with animals fed vegetable oils. In the same species a high-fat diet containing margarine had no effect on the serum cholesterol (PELTONEN & KARVONEN 1956).

In the chick little or no change in serum cholesterol or atheromatosis has been observed after feeding cottonseed oil (DAUBER & KATZ 1943, PETERSON & HIRST 1951), lard (PETERSON & HIRST 1951), cod-liver oil or linseed oil (DAM et al. 1959) without supplementary cholesterol. In cholesterol-fed animals cottonseed oil enhanced the serum cholesterol more than did corn oil (JONES et al. 1956) and linseed oil raised the cholesterol level more than did cod-liver oil (DAM et al. 1959). Little or no difference in hypercholesterolemia and atherogenesis was produced by dietary fats with varying degree of saturation, such as coconut, peanut or sardine oil (KING et al. 1956), by feeding saturated animal fat *e. g.* lard and egg yolk, versus unsaturated vegetable oils, such as cottonseed and corn oil (STAMLER et al. 1957), or by substitution of corn oil for butter fat at the 10 % level (STAMLER et al. 1959). In contrast hereto, KUMMEROW (1955) found that cholesterol-fed chickens had higher blood cholesterol levels when the dietary fat consisted of lard, butter fat or hydrogenated cottonseed oil than when highly unsaturated cottonseed oil was given.

Very recently BRECHTER using a cholesterol-free semi-synthetic basal diet found that coconut oil or butter fat fed cockerels had significantly higher serum cholesterol than animals fed corn oil (personal communication).

In swine fed 40 % butter fat in a cholesterol-free diet the serum cholesterol levels observed were higher than in animals on 40 % corn oil (BRAGDON et al. 1957) or on 40 % margarine (ROWSELL et al. 1958).

In experiments on dogs TSAI et al. (1954) found that animals first fed on a commercial diet and then on experimental diets containing 13 % fat entirely of vegetable origin showed a fall in serum cholesterol, despite the fact that the experimental diet contained more than twice and a half as much fat as the control diet.

Also these results obtained in experiments with species other than rabbits

indicate that, under certain experimental conditions, different dietary fats might influence lipid metabolism and the appearance of the aorta in different ways.

The present results showed that in the rabbits fed butter fat, hydrogenated coconut oil or glyceryl trilaurate a distinct increase in the serum cholesterol generally occurred when the animals had been on the experimental diet for 6 weeks. However, in a few of the animals a relatively long period elapsed before the serum cholesterol definitely began to rise. Such kind of "resistance" has been observed in cholesterol-fed rabbits by FILLIOS & MANN (1956) far more frequently in male than in female animals, and they ascribed it in part to sex variability. With proper Estradiol treatment "resistant" male rabbits could be made to respond, while "susceptible" females could be made more resistant by administration of testosterone. A rather wide fluctuation of the lipid levels in serum was observed throughout the feeding periods, as has been found in cholesterol-fed rabbits (WANG et al. 1954, MANN & STARE 1955).

The low cholesterol levels found on feeding fluid vegetable oils were accompanied by significant weight gain of the animals. On the other hand, hypercholesterolemia was most pronounced in the groups fed hydrogenated coconut oil or lauric acid, several animals in these groups showing a moderate weight loss. Supplements of linoleic acid or corn oil to the lauric acid diet appeared to improve weight gain. These latter findings are in agreement with those reported by FUNCH et al. (1957) in newly weaned rats fed 7 % or 28 % trilaurate with or without supplements of 20—100 mg ethyl-linoleate. In the present experiments supplements of linoleic acid or corn oil also appeared to modify the striking cholesterol-enhancing effect of lauric acid. Somewhat similar results have been reported by TAUPITZ & WIETEK (1957), who found lower cholesterol concentrations in serum, liver and aorta of cholesterol-fed rabbits, when the green-fodder diet was supplemented with a mixture of linoleic and linolenic acid.

The ratio between total cholesterol and phospholipids in the serum has been considered of importance for the stability of the serum lipids (AHRENS & KUNKEL 1949, LADD et al. 1949, GERTLER et al. 1950) and has been found to be increased in survivors of myocardial infarction, as examples of advanced atherosclerosis (BARR 1953) as well as in rabbits with cholesterol-induced hypercholesterolemia and atherosclerosis (LADD et al. 1949, WANG et al. 1954, DURY 1957). At the end of the experiments in the present investigation the highest ratio was found in the groups fed butter fat or hydrogenated coconut oil. A statistically significant positive correlation existed between this ratio and the incidence of atherosclerotic changes in the aorta.

It has been suggested that the concentration of triglycerides in serum may possibly be an important factor in atherogenesis (ANFINSEN 1955). Recently ALBRINK & MAN (1959) found elevated serum triglyceride levels in 85—90 % of patients with coronary artery disease. It is evident from the present investigation that while the atherosclerotic changes observed in the coconut oil group were accompanied by high serum triglyceride levels and lactescence of the serum, aortic changes occurred even with essentially normal triglyceride values, as seen in the animals fed butter fat. On the other hand, both the corn oil and the rapeseed oil groups showed a significant fall in the serum triglycerides. In these groups no aortic lesions were detectable. While cholesterol-fed rabbits usually show an increase in serum neutral fat and lactescence (SCHÖNHEIMER 1924, FRIEDMAN & BYERS 1954, WANG et al. 1954, DURY 1957), COX et al. (1958) found hypercholesterolemia in Rhesus monkeys fed butter fat and cholesterol but no increase in the serum neutral fat or lactescence of the serum. In our series, rabbits that received lauric acid as the only dietary source of fat had high triglyceride levels and milky serum, while lower values and clear serum were found in the animals that received supplements of either linoleic acid or corn oil.

It is clear from the results that the determination of the serum total lipid content alone may lead to erroneous conclusions on the effect on the lipid metabolism of the dietary fat to be tested. Thus, though the total lipid level before and at the end of the feeding periods was unchanged in the corn oil and rapeseed oil groups, significant changes nevertheless occurred in the different lipid fractions. On the other hand, feeding butter fat resulted in a marked increase of the total lipid, while the triglyceride concentration remained essentially unchanged.

In recent years attention has been focused on certain protein-lipid relationships in serum and their importance in the pathogenesis of human atherosclerosis and its early diagnosis (GOFMAN et al. 1950). Raised β-lipoprotein levels have been found in patients who have survived coronary occlusion (BARR et al. 1951) and in patients with various forms of hypercholesterolemia (SWAHN 1953). In the present investigation feeding butter fat or hydrogenated coconut oil produced a striking relative increase in the serum $\beta+0$-lipoproteins of the same order as that described by BOSSAK et al. (1954) in cholesterol-fed rabbits. On the other hand, corn oil and rapeseed oil produced no significant changes. POLLAK (1956), who also found a considerable increase in this fraction in rabbits fed cholesterol together with cottonseed oil, noted that feeding oil alone had little influence, the β-lipoproteins showing only a transient increase.

An equal distribution of the different lipid fractions throughout the liver

was found, which is in agreement with the findings in human liver reported by Rourke & Stewart (1942) and Billing et al. (1953). Feeding butter fat or hydrogenated coconut oil produced a marked increase in the ester-bound cholesterol, which, on the other hand, rose only slightly when corn oil or rapeseed oil was fed. The free cholesterol remained fairly constant with the exception of the coconut oil group, which showed a slight increase in this fraction.

Striking differences in the triglyceride content in liver were found. While corn oil and rapeseed oil significantly depressed the triglyceride level, butter fat resulted in an accumulation of this fraction, which was, however, not of such an order as to produce excessively fatty livers. Hydrogenated coconut oil, on the other hand, produced no statistically significant changes. In studies on rabbits fed a 40 % sunflowerseed meal diet Aylward & Stott (1937) observed an initial increase in the liver triglycerides, which then dropped to values only slightly above normal. The livers showed no excessive fatty changes despite the fact that the animals received 25 g fat per day. The percentages of phospholipid, free cholesterol and ester-bound cholesterol did not vary appreciably from normal. Sveinsson (1937) observed that green fodder diets containing 25—40 % of coco fat fed to rabbits produced livers containing up to 30—40 % of fatty acids.

The phospholipid fraction was found to comprise the largest portion of the total lipid content in the liver, this finding being in agreement with the result of Page et al. (1931), Aylward & Stott (1937), MacLachlan (1940), Weinhouse & Hirsch (1940) and Dury (1957). Compared with the other lipids, the phospholipids, like the free cholesterol, showed no or only moderate changes. This is in agreement with observations made in cholesterol-fed rabbits (Dury 1957) and in rabbits fed a high-fat diet in the form of 40 % sunflowerseed meal (Aylward & Stott 1937) as well as in cholesterol-fed rats (Ridout et al. 1954, Deuel 1955).

Systematic investigations on the influence of different dietary fats on the liver lipids seem to have been made mainly on rats. The earlier results are extensively summarized by Hartroft (1955). More recently there has been found an accumulation of liver cholesterol with increasing degree of unsaturation of the dietary fat (Brown & Lewis 1956, Diller et al. 1957, Best et al. 1958, Okey et al. 1959). On the other hand, feeding relatively saturated fats, such as butter, beef fat, lard, and coconut oil has been reported to raise the liver lipid levels more than less saturated oils, such as corn oil or cod-liver oil (Channon & Wilkinson 1936, Benton et al. 1956, Avigan & Steinberg 1957). Alfin-Slater et al. (1954) found an increased esterified cholesterol concentration in the liver of essential fatty acid-deficient male rats.

In chicks liver cholesterol levels have been found to be higher when the diet was fat-free than when it contained 3 %, 10 % or 20 % unhydrogenated peanut oil but no cholesterol (DAM et al. 1955, 1956). Feeding 10 % or 20 % of hydrogenated peanut oil, on the other hand, did not result in such a difference (DAM et al. 1958). Without dietary cholesterol no difference in liver cholesterol was found between cod-liver oil fed and linseed oil fed chicks. However, with 1 % dietary cholesterol linseed oil caused higher levels than did cod-liver oil (DAM et al. 1959).

These results as well as those from investigations on serum lipids and atherogenesis obtained in various species and mentioned on page 66 have been contradictory and are not strictly comparable with those obtained in the rabbit. Besides the differences in species, the experimental designs used have differed regarding the breed, age, sex, body-weight of the animals and the composition and cholesterol content of the basal diet, factors which probably influence the response to the dietary fat tested.

The determination of the lipid excretion in feces showed that the differences in the effect of the different fats fed on the serum and liver lipid partition were not due to variations in the fat absorption. The absorption percentage of the different fats fed was found to be over 90 %. Rapeseed oil showed the lowest absorption percentage, which is in agreement with the finding of THOMASSON & GOTTENBOS (1957), who observed somewhat poorer absorption of rapeseed oil compared with butter fat, coconut oil or corn oil in rats. Some of the rabbits fed hydrogenated coconut oil or lauric acid lost weight, while all other animals showed weight gain. It may be mentioned that the animals in these two groups did not consume all their tablets so soon as the animals in the other groups. Studying the rates of absorption of 18 natural oils and fats of vegetable and animal origin in rats THOMASSON (1956) found a significant correlation between the rate of absorption and the growth action of these oils and fats.

At the end of the feeding periods a somewhat larger excretion of the non-saponifiable fraction was noted in the corn oil and rapeseed oil groups, which had low serum cholesterol levels, compared with the butter fat and coconut oil groups, where hypercholesterolemia was the rule. The non-saponifiable fraction in the fecal lipids consists mainly of cholesterol and coprosterol (DEUEL 1955) and may be taken as a measure of cholesterol (COOK & THOMSON 1950). The somewhat greater excretion in the groups fed fluid vegetable oils may have depended, at least partly, upon the higher content of phytosterols in these oils. These sterols have been found to be less readily absorbed than cholesterol (GOULD et al. 1956, SWELL et al. 1958). On the other hand, GORDON et al. (1957) and HELLMAN et al. (1957) have

suggested that the cholesterol-depressing effect of the vegetable oils might be due, at least in part, to an increase in the excretion of the sterol from the body. In this connection the finding of WILSON & SIPERSTEIN (1958) is of interest that in rats feeding of diets containing 30 % corn oil resulted in a marked acceleration in excretion of non-digitonin precipitable neutral sterol as compared with diets containing lard or no fat.

Most of the rabbits fed hydrogenated coconut oil or butter fat showed a progressive loss of hair during the four months of the feeding period. According to gas-chromatographic analysis, this diet was free or almost free of polyunsaturated fatty acid. The changes in the fur observed might therefore have been a manifestation of essential fatty acid deficiency. This suggestion is supported by the finding that no such changes were observed in the groups fed vegetable oils, which had a high polyunsaturated acid content. Further, while loss of hair and poor body-growth were also observed in those rabbits that received pure glyceryl trilaurate, feeding the same diet, but with an addition of linoleic acid or corn oil, produced no such symptoms. PEIFER & HOLMAN (1956) have shown that rabbits on an essential fatty acid-free diet containing 1 % cholesterol became depleted of these acids in 3 months, as evidenced by loss of hair, emaciation and skin lesions. These symptoms resemble the EFA-deficiency syndrome originally described by BURR & BURR (1929) in rats. Administration of the polyunsaturated fatty acids linoleic (BURR et al. 1932), linolenic (BURR et al. 1932), or arachidonic acid (HUME et al. 1940), was reported capable of preventing and curing this disease, while none of the saturated fatty acids occurring in hydrogenated coconut oil were effective in this respect (BURR & BURR 1930). Later, BURR et al. (1940) showed that while linoleic and arachidonic acid cured the skin lesions linolenic acid had little or no such effect on the skin, though this fatty acid gave good growth response.

The EFA-syndrome has since been described in other species, such as mice (WHITE et al. 1943), guinea-pigs (REID 1954), dogs (HANSEN & WIESE 1943, 1951), chickens (REISER 1950), calves (LAMBERT et al. 1954), and swine (HILL et al. 1957), but, as a rule, young growing animals have been used for the induction of the fully developed syndrome. In the present investigation, in which adult male rabbits were used, loss of hair was the only dermal symptom, which is in agreement with the findings of LAMBERT et al. (1958) in adult male rabbits fed a 20 % hydrogenated coconut oil diet. In some of the present rabbits kept on the coconut oil diet for one year the loss of hair disappeared spontaneously without any change in the diet. BARKI et al. (1947) noted spontaneous disappearance of dermal EFA-deficiency symptoms in mature rats maintained at first on a restricted fat-free diet and then

supplied the same diet *ad lib.* for sufficiently long periods, *i. e.* 66—154 days. AAES-JØRGENSEN et al. (1958) found that it was difficult and required a long time to induce visible signs of EFA-deficiency in adult male rats. These authors also noted spontaneous cures of the dermal disorder in the animals on the EFA-free diets before the 52nd week. The underlying mechanism of this tendency to spontaneous healing is not clear. Evidence has accumulated that — in contrast to what had been indicated by earlier reports (BERNHARD & SCHÖNHEIMER 1940, BERNHARD et al. 1942, SCHÖNHEIMER 1946) — synthesis of essential fatty acids takes place to a certain extent in the animal's body (MEDES & KELLER 1947, BARKI et al. 1947, 1949), but not in sufficient quantity to satisfy the animal's needs (HOLMAN 1954). In the present investigation no definite correlation was found between the fur changes and loss of body-weight.

The gross aortic changes observed in most of the rabbits fed butter fat, hydrogenated coconut oil or glyceryl trilaurate resembled the typical picture of experimental atherosclerosis described by LEARY (1934, 1949), DUFF (1935), POLLAK (1945), and RANNIE & DUGUID (1953) in cholesterol-fed rabbits. Although the lesions were, as a rule, of moderate severity, in some cases they were fairly advanced, particularly in rabbits fed hydrogenated coconut oil for 15 weeks. The most advanced lesions were found in a rabbit fed hydrogenated coconut oil for one year.

No lipid deposits were seen in the aorta of the animals fed corn oil or rapeseed oil. This is in agreement with the negative results obtained in the experiments mentioned on page 63, in which vegetable oils had been used in cholesterol-free diets. On the other hand, it does not confirm the findings by STEINER & DAYTON (1956) and LAMBERT et al. (1958) that feeding unsaturated vegetable fat produced aortic changes in some of the rabbits studied. In these last-mentioned experiments high-fat rations were fed.

The histological description is not part of the present investigation and will be published elsewhere (STORMBY & WIGAND, in preparation). It may be mentioned already here that no microscopic aortic changes were found in the control group or the groups fed fluid vegetable oil. It is not possible, on the basis of the gross appearance of the aortic changes alone to draw any definite conclusions regarding the similarities and differences between experimental and human atherosclerosis. This subject is dealt with in detail by LEARY (1934), DUFF (1935) and ALTSCHUL (1950 a).

The severity of the aortic changes was significantly correlated with the cholesterol level and the magnitude of the cholesterol/phospholipid ratio in serum. On the other hand, judging from the entire material, no correlation appeared to exist between the aortic changes and the triglyceride content of

the serum, since aortic lesions developed in the butter fat group, where the triglyceride content did not differ from that in the controls on an ordinary diet. Neither did any correlation appear to exist between the aortic changes and the severity of hair loss.

It has been claimed that atherosclerosis does not occur spontaneously in rabbits (DUFF 1936). However, recently BRAGDON (1952) found microscopic lesions interpreted as representing early atherosclerosis in the aortae of most suckling rabbits. This is a period characterized by relatively high plasma lipid levels. In the postweaning period the lesions regressed. This author claimed that deposition of lipid also occurred in the older animal. We have not seen any such changes in the control group. Nor were any spontaneous medial changes observed of the type described by HEPTINSTALL & BRONTE-STEWART (1954).

As to the factors responsible for the effects exerted by the different dietary fats, no valid explanation can be offered on the basis of the present investigation. However, some facts, which may be taken as a point of departure for further investigations have emerged. Thus, hydrogenated coconut oil, which exerted the strongest hypercholesterolemic and atherogenic effect, together with dermal symptoms, was completely devoid of polyunsaturated — and essential — fatty acids. Oils, such as corn oil and rapeseed oil, which were rich in polyunsaturated fatty acids, on the other hand, had hardly any enhancing effect on the serum cholesterol level and no atherosclerosis occurred in these groups. Rapeseed oil contained about 50 % erucic acid. This acid has been found to have no effect on normal blood cholesterol in rats (CARROLL & NOBLE 1956). The coconut oil used contained 30 % of the medium chain fatty acid, lauric acid, which, when fed alone, increased the serum cholesterol to comparable levels, as did the whole oil and to definitely higher values than has been observed on feeding a fat-free semi-synthetic diet for 24 weeks (MALMROS & WIGAND 1959). The cholesterol-enhancing effect of lauric acid appeared to be modified to a certain degree by supplements of pure linoleic acid or corn oil.

Butter fat produced both hypercholesterolemia and atherosclerosis. This fat contained only a small amount polyunsaturated fatty acids. However, it contained about 0.3 % cholesterol providing a daily intake of about 7 mg per kg body-weight of this potent hypercholesterolemic sterol.

Corn oil and rapeseed oil had a somewhat higher content of phytosterols than coconut oil. It cannot be excluded that this might have contributed to the low serum cholesterol values observed in these groups (BEVERIDGE et al. 1956, 1957, GRANDE et al. 1958) by interfering with the reabsorption of excreted cholesterol (PETERSON 1958). A report that plant sterols are able

to produce atherosclerosis in rabbits (Curran & Costello 1956) could not be confirmed by Beher et al. 1957 and Shipley et al. (1958), who fed rabbits on plant sterols for a much longer time. Strong evidence has accumulated that it is the fatty acid fraction in vegetable oils that is responsible for most if not all of the cholesterol-depressant effect of these oils and that the non-saponifiable fraction has no activity in this respect (Ahrens et al. 1957, Bronte-Stewart 1958, De Groot & Reed 1959, Worne & Smith 1959).

Chapter VII

GENERAL SUMMARY

Clinical research during the last few years has shown that the nature of dietary fat influences lipid metabolism in man much more than the amount of fat ingested. In the investigation of any atherogenic property of different dietary fats research must resort to animal experiments.

In the present investigation a bio-assay has been described, which may be suitable for such experiments. Adult male rabbits were fed for 15—18 weeks on a cholesterol-free, semi-synthetic diet, the fat to be studied being added at the 8 % level by weight, which corresponds to 26 % of the calories.

The lipid partition in serum and liver and the incidence of gross atherosclerosis in the aorta were studied in rabbits fed four different fats and oils, namely butter fat, hydrogenated coconut oil, corn oil or rapeseed oil. In addition, a few animals were fed 8 % glyceryl trilaurate with or without a supplement of 4 % linoleic acid or corn oil. The following observations were made:

1. All of the animals increased moderately in weight except those fed hydrogenated coconut oil or glyceryl trilaurate, some of which showed a moderate loss of body-weight.

2. In control animals fed *ad lib.* on a diet consisting of crushed barley and green roughage respectively mangels for 18 weeks no change was observed in the lipid partition in serum except a slight decrease in the free cholesterol.

3. Feeding rabbits on a diet containing butter fat as the fat source regularly produced hyperlipemia with an increase in both the free and particularly in the ester-bound cholesterol and a somewhat smaller increase in the phospholipids resulting in an increase in the cholesterol/phospholipid ratio. On the other hand, the triglyceride level remained unchanged. A marked increase was noted in the $\beta+0$-lipoproteins in serum.

Compared with the controls, a pronounced increase occurred in the ester-bound cholesterol in the liver and an accumulation of triglycerides, while the phospholipid concentration showed only a moderate increase and the free cholesterol remained essentially normal.

4. Feeding hydrogenated coconut oil resulted in a hyperlipemia that was more pronounced than in the butter fat fed animals. In this group the free and the ester-bound cholesterol increased to the same extent together with a somewhat smaller increase in the phospholipids, which resulted in an increase of the cholesterol/phospholipid ratio. A marked increase in the serum triglyceride levels occurred. The increase in the $\beta+0$-lipoproteins in the serum was of the same order as that seen in the butter fat group.

The liver showed a definite increase in the ester-bound cholesterol but only a slight increase in the free cholesterol. The triglyceride content was not changed, while the phospholipid concentration was moderately decreased.

5. Unlike butter fat and hydrogenated coconut oil, corn oil produced only a slight increase in both the free and ester-bound cholesterol and the phospholipids in the serum. The cholesterol/phospholipid ratio increased. A distinct fall was noted in the serum triglyceride content. The level of the $\beta+0$-lipoproteins persisted unchanged.

In the liver the ester cholesterol fraction showed a slight rise, while a distinct decrease occurred in the triglyceride fraction. The phospholipid level was moderately lower than in the control group.

6. In the group fed rapeseed oil a slight increase occurred in the ester-bound cholesterol in serum, while the free cholesterol remained unchanged. Also in this group a distinct decrease was noted in the triglyceride concentration, while the phospholipid content remained unchanged. The cholesterol/phospholipid ratio increased. No significant change occurred in the level of the $\beta+0$-lipoproteins in serum.

Only a moderate increase was noted in the ester-bound cholesterol in the liver, while the free cholesterol remained unchanged. The phospholipid fraction was the same as in the control group, while the triglyceride fraction was distinctly decreased.

7. The hypercholesterolemic effect of hydrogenated coconut oil continued during a feeding period of 54 weeks in 3 rabbits studied. Replacement of hydrogenated coconut oil by corn oil in 2 other animals after 21 weeks resulted in a dramatic fall in the serum cholesterol and a gradual return to normal values.

8. Feeding 8 % glyceryl trilaurate produced a pronounced increase in the concentration of cholesterol, triglycerides, phospholipids and $\beta+0$-lipoproteins in the serum, as well as of the ester-cholesterol fraction in the liver of 4 rabbits studied. Addition to this diet of 4 % linoleic acid or corn oil appeared to modify this effect of lauric acid in another 4 animals studied.

9. The absorption percentage of the different fats and oils used was found to be between 94 % and 100 %. The excretion of the non-saponifiable fraction

in the feces was somewhat higher in the rabbits fed corn oil or rapeseed oil, compared with the butter fat or coconut oil fed animals.

10. Feeding butter fat, hydrogenated coconut oil or glyceryl trilaurate resulted in loss of hair in most of the animals suggesting essential fatty acid deficiency. In 2 of 3 adult rabbits fed hydrogenated coconut oil for one year these changes regressed spontaneously towards the end of the feeding period.

11. Gross atherosclerotic lesions in the form of sudanophilic plaques in the aorta were observed in 12 of 13 rabbits fed butter fat, in 16 of 18 rabbits fed hydrogenated coconut oil and in all of the 4 rabbits fed glyceryl trilaurate. Animals fed corn oil, rapeseed oil or green roughage and barley showed no aortic changes.

12. A significant positive correlation was found between the frequency and severity of the aortic lesions and the content of the free, ester-bound and total cholesterol as well as the total cholesterol/phospholipid ratio in the serum.

The results presented above have shown that it is possible to produce gross atherosclerotic lesions in the aorta of adult male rabbits by feeding them on a cholesterol-free, semi-synthetic basal diet, to which hydrogenated coconut oil or butter fat is added as 8 % by weight corresponding to 26 % of the calories supplied. Comparison between these two fats and fluid vegetable oils, such as corn oil and rapeseed oil revealed that, under the experimental conditions used, different dietary fats may differ profoundly in their effect on the lipid partition in serum and liver and that they may have quite different atherogenic properties.

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